PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C07K 1/04, 5/02 | A1

(11) International Publication Number: WO 96/22304

(43) International Publication Date: 25 July 1996 (25.07.96)

(21) International Application Number: PCT/US96/00786

(22) International Filing Date: 19 January 1996 (19.01.96)

(30) Priority Data:
08/375,904 20 January 1995 (20.01.95) US

(71) Applicant: MOLECUMETICS, LTD. [US/US]; Suite 400, 2023 - 120th Avenue N.E., Bellevue, WA 98005-2189 (US).

(72) Inventor: KAHN, Michael; 10916 80th Place N.E., Kirkland, WA 98034 (US).

(74) Agents: HERMANNS, Karl, R. et al.; Seed and Berry L.L.P., 6300 Columbia Center, 701 Fifth Avenue, Seattle, WA 98104-7092 (US).

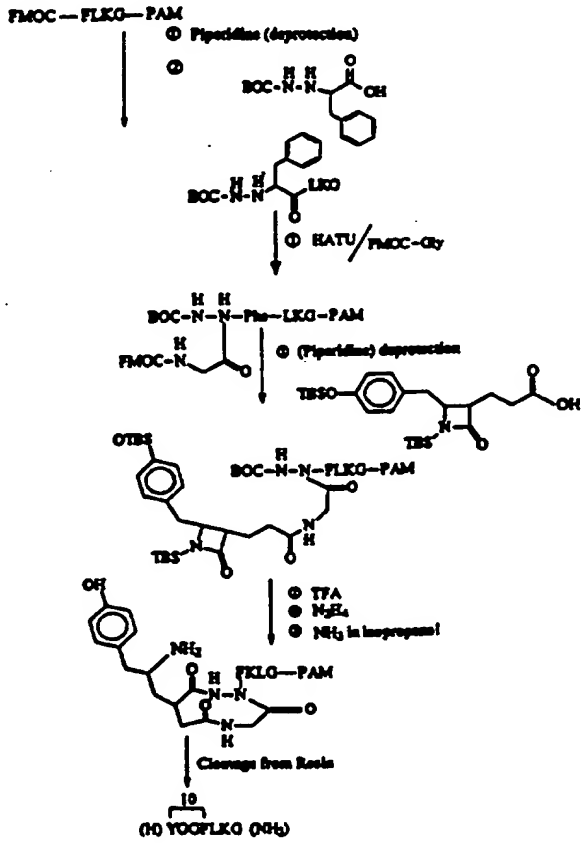
(81) Designated States: AL, AM, AU, AZ, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, RO, RU, SD, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AZ, BY, KG, KZ, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: CONFORMATIONALLY CONSTRAINED REVERSE-TURN LIBRARY AND METHODS RELATING THERETO

(57) Abstract

Libraries of conformationally constrained-reverse turn mimetics, and methods of constructing the same are disclosed. The reverse-turn mimetics include beta-turn, gamma-turn and beta-bulge mimetics which mimic the biologically active conformation of a linear peptide. In a preferred embodiment, a template library is constructed based on the amino acid sequence of the linear peptide, and which contains a plurality of beta-turn, gamma-turn and beta-bulge mimetics of varying ring sizes. The template library is then screened in a suitable assay to identify a biologically active template library member. An optimized library is then constructed based on structure of the biologically active template member, and which includes substitutions to the amino acid sequence thereof. The optimized library is similarly screened to identify a biologically active reverse-turn mimetic. Methods relating to identifying library members which interact with biological targets of interest, and the use of such members as diagnostic, prophylactic and/or therapeutic agents are also disclosed.



FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	**GB**	United Kingdom	**MW**	Malawi
AT	Austria	**GE**	Georgia	**MX**	Mexico
AU	Australia	**GN**	Guinea	**NE**	Niger
BB	Barbados	**GR**	Greece	**NL**	Netherlands
BE	Belgium	**HU**	Hungary	**NO**	Norway
BF	Burkina Faso	**IE**	Ireland	**NZ**	New Zealand
BG	Bulgaria	**IT**	Italy	**PL**	Poland
BJ	Benin	**JP**	Japan	**PT**	Portugal
BR	Brazil	**KE**	Kenya	**RO**	Romania
BY	Belarus	**KG**	Kyrgystan	**RU**	Russian Federation
CA	Canada	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CF	Central African Republic			**SE**	Sweden
CG	Congo	**KR**	Republic of Korea	**SG**	Singapore
CH	Switzerland	**KZ**	Kazakhstan	**SI**	Slovenia
CI	Côte d'Ivoire	**LI**	Liechtenstein	**SK**	Slovakia
CM	Cameroon	**LK**	Sri Lanka	**SN**	Senegal
CN	China	**LR**	Liberia	**SZ**	Swaziland
CS	Czechoslovakia	**LT**	Lithuania	**TD**	Chad
CZ	Czech Republic	**LU**	Luxembourg	**TG**	Togo
DE	Germany	**LV**	Latvia	**TJ**	Tajikistan
DK	Denmark	**MC**	Monaco	**TT**	Trinidad and Tobago
EE	Estonia	**MD**	Republic of Moldova	**UA**	Ukraine
ES	Spain	**MG**	Madagascar	**UG**	Uganda
FI	Finland	**ML**	Mali	**US**	United States of America
FR	France	**MN**	Mongolia	**UZ**	Uzbekistan
GA	Gabon	**MR**	Mauritania	**VN**	Viet Nam

Description

CONFORMATIONALLY CONSTRAINED REVERSE-TURN LIBRARY AND METHODS RELATING THERETO

Related Applications

This application is a continuation-in-part of U.S.S.N. 08/213,124, filed March 15, 1994; and a continuation-in-part of U.S.S.N. 08/236,674, filed May 2, 1994, which is a continuation of 07/926,350, filed August 6, 1992, which is a continuation-in-part of U.S.S.N. 07/651,800, filed February 7, 1991.

Technical Field

This invention relates generally to libraries of conformationally constrained reverse-turn mimetics, methods relating to the identification of library members that interact with biological targets of interest, and the use of such members as diagnostic, prophylactic and/or therapeutic agents.

Background of the Invention

Random screening of molecules for possible activity as therapeutic agents has occurred for many years and resulted in a number of important drug discoveries. While advances in molecular biology and computational chemistry have led to increased interest in what has been termed "rational drug design", such techniques have not proven as fast or reliable as initially predicted. Thus, in recent years there has been a renewed interest and return to random drug screening. To this end, particular strides having been made in new technologies based on the development of combinatorial chemistry libraries, and the screening of such libraries in search for biologically active members.

In general, combinatorial chemistry libraries are simply a collection of molecules. Such libraries vary by the chemical species within the library, as well as the methods employed to both generate the library members and identify which members interact with biological targets of interest. While this field is still young, methods for generating and screening libraries have already become quite diverse and sophisticated. For example, a recent review of various combinatorial chemical libraries has identified a number of such techniques, including the use of both tagged and untagged library members (Janda, *Proc. Natl. Acad. Sci. USA* 91:10779-10785, 1994).

To date, combinatorial chemistry libraries have generally been limited to members of peptide or nucleotide origin. To this end, the techniques of Houghten et al.

illustrate an example of what is term a "dual-defined iterative" method to assem soluble combinatorial peptide libraries via split synthesis techniques (*Nature (Lond* 354:84-86, 1991; *Biotechniques* 13:412-421, 1992; *Bioorg. Med. Chem. Lett.* 3:4 412, 1993). By this technique, soluble peptide libraries containing tens of millions members have been obtained. Such libraries have been shown to be effective in identification of opioid peptides, such as methionine- and leucine-enkephalin (Doo and Houghton, *Life Sci.* 52, 1509-1517, 1993), and a N-acylated peptide library l been used to identify acetalins, which are potent opioid antagonists (Dooley et al., *Pr Natl. Acad. Sci. USA* 90:10811-10815, 1993). More recently, an all D-amino a opioid peptide library has been constructed and screened for analgesic activity agai the mu ("μ") opioid receptor (Dooley et al, *Science* 266:2019-2022, 1994).

While combinatorial libraries containing members of peptide a nucleotide origin are of significant value, there is still a need in the art for librar containing members of different origin. For example, traditional peptide libraries tc large extent merely vary the amino acid sequence to generate library members, and not impart a constrained secondary structure to such peptide members. It is w recognized that the secondary structures of peptides are important to biological activi Thus, there is a need in the art for libraries containing members which mimic the act structure of biologically active peptides. There is also a need in the art for methods generating such libraries, as well as techniques for screening the library members agair targets of interest, particularly biological targets, to identify bioactive library membe The present invention fulfills these needs, and provides further related advantages.

Summary of Invention

Briefly stated, the present invention is directed to libraries conformationally constrained reverse-turn mimetics and methods relating to t generation thereof. This invention is further directed to methods relating to t screening and identification of library members that interact with biological targets interest, as well as the use of such identified members as diagnostic, prophylactic and/ therapeutic agents.

In one embodiment of this invention, a library containing a plurality conformationally-constrained reverse-turn mimetics as members is disclosed, t structures of which are set forth in greater detail in the following detailed description. a related aspect, a method for identifying biologically active agents is disclose comprising screening the above library members in a suitable assay, and thereb identifying biologically active library members.

In a further embodiment, there is disclosed a method for identifying a biologically active conformationally constrained reverse-turn mimetic which mimics a biologically active conformation of a linear peptide having a known amino acid sequence. In this method, conformationally constrained reverse-turn mimetics are constructed based on the known amino acid sequence of the linear peptide to yield a template library containing a plurality of members selected from conformationally constrained beta-turn, gamma-turn and beta-bulge mimetics having multiple ring sizes. The template library members are then screened in a suitable assay to identify a biologically active member of the template library. Next, conformationally constrained reverse-turn mimetics are constructed based on the biologically active member of the template library to yield an optimized library containing a plurality of members having varying amino acid substitutions based on the known amino acid sequence of the biologically active linear peptide. Lastly, the optimized library members are screened in a suitable assay to identify a biologically active member of the optimized library.

In yet a further embodiment, conformationally constrained reverse-turn mimetics are disclosed which are useful as diagnostic, prophylactic and/or therapeutic agents. The structure of such mimetics, as well as methods to synthesis the same, are disclosed in greater detail in the following detailed description.

Other aspects of this invention will become apparent upon reference to the following detailed description.

Brief Description of the Drawings

Figures 1A-1B illustrate a representative synthesis of conformationally constrained beta-turn mimetics having 12- and 14-membered rings.

Figures 2A-2C illustrate a representative synthesis of a conformationally constrained beta-turn mimetic having a 10-membered ring, as well as a gamma-turn mimetic having a 7-membered ring.

Detailed Description of the Invention

The present invention is generally directed to libraries containing conformationally constrained reverse-turn mimetics of naturally occurring or synthetic peptides (referred to herein as "reverse-turn mimetics" or "mimetics"), and which are useful in the identification of bioactive agents, such as diagnostic, prophylactic and/or therapeutic agents. In the practice of the present invention, the libraries may contain from tens to hundreds to even millions of individual reverse-turn mimetics (also referred to herein as "members").

Once assembled, the libraries of the present invention may be screenec identify individual members having bioactivity. Such screening of the libraries bioactive members may involve, for example, evaluating the binding activity of members of the library or evaluating the effect the library members have on a functic assay. Screening is normally accomplished by contacting the library members (o subset of library members) with a target of interest, such as, for example, an antibo enzyme, receptor or cell line. Library members which are capable of interacting with target of interest are referred to herein as "bioactive library members" or "bioact mimetics". For example, a bioactive mimetic may be a library member which is capa of binding to an antibody or receptor, which is capable of inhibiting an enzyme, or wh is capable of eliciting or antagonizing a functional response associated, for example cell line. In other words, the screening of the libraries of the present invent determines which library members are capable of interacting with one or more biologi targets of interest. Furthermore, when interaction does occur, the bioactive mimetic mimetics) may then be identified from the library members. The identification of a sin (or limited number) of bioactive mimetic(s) from the library yields reverse-turn mimet which are themselves biologically active, and thus useful as diagnostic, prophylactic therapeutic agents, and may further be used to significantly advance identification of le compounds in these fields.

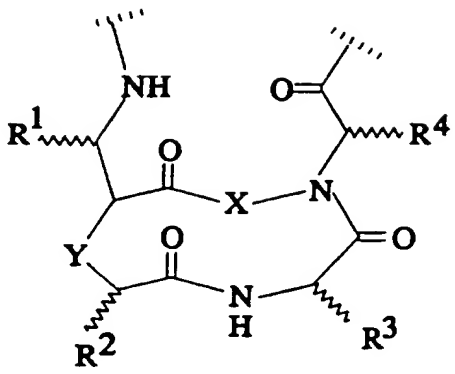
The steps which are undertaken to identify a bioactive mimetic from t library depends primarily on whether the library is spatially or nonspatially addressab While either library type is permitted in the practice of this invention, spatia addressable libraries are preferred. In a spatially addressable library, each reaction ves in which an assay is performed contains a single mimetic or a small number of mimeti Thus, in a spatially addressable library, when a bioactive mimetic is identified in screening assay, its identity is specifically known, or known to be of a limited number mimetics. In general, the size of spatially addressable libraries are limited by the requir degree of spatial resolution. However, significant advances have recently been ma which permit a relatively large number of members within a spatially addressable libra For example, photolithographic techniques now allow for the spatial resolution relatively large number of members (on the order of 64,000) in a small area, such as 1.5 cm^2 chip. (Fodor et al., *Science* 251:717-723, 1991; Cho et al., *Science* 261:13C 1305, 1993) (both of which are incorporated by reference herein). Such techniques a known to those skilled in the art and summarized in Janda, *Proc. Natl. Acad. Sci. U.* 91:10779-10785, 1993 (incorporated herein by reference). Large, spatially addressab

libraries may also be constructed using an iterative approach, as disclosed by, for example, Houghten et al. (*Nature* 354:84-86, 1991) (incorporated herein by reference).

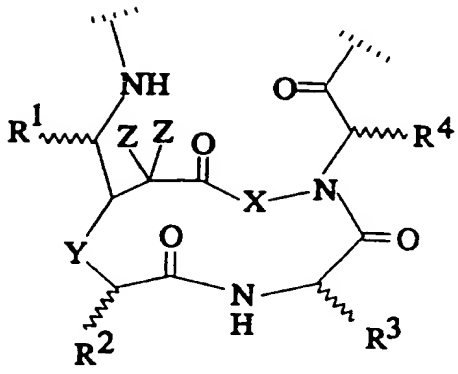
Alternatively, nonspatially addressable libraries can be made by constructing an encoded library as disclosed by Ohlemeyer et al. (*Proc. Natl. Acad. Sci. USA* 90:10922-10926, 1993) and Brenner et al. (*Proc. Natl. Acad. Sci. USA* 89:5181-5183, 1992) (both of which are incorporated herein by reference). In short, a nonspatially addressable library requires the ability to directly sequence the minute quantity of product that is associated with a polymer bead in the synthesis. This is readily achievable at the 5-10 picomolar range with peptide sequencing. Alternative, a nonsequenceable number can be cosynthesized with a coding sequenceable partner (*e.g.*, peptide or oligonucleotide) which can be directly sequenced, or PCRed and sequenced. Small sequenceable coding strands can be made which can be sequenced using mass spectroscopy (*see, e.g.*, Ohlemeyer et al., Nikolaiev et al., *Peptide Res.* 6:161-170, 1993) (incorporated herein by reference).

In one aspect of this invention, methods for the synthesis of library members are disclosed. As mentioned above, the mimetics of the present invention comprise conformationally constrained reverse-turn mimetics and, more specifically, encompass compounds of related structures, including mimetics of beta-turns (β-turns), gamma turns (γ-turns), and beta-bulges (β-bulges). In general, beta-turns are reversals in the direction of a polypeptide chain wherein the oxygen of the CO group of amino acid *n* is hydrogen bonded to the hydrogen of the NH group of amino acid *n+3*. Similarly, gamma-turns and beta-bulges are reversals in a polypeptide chain where the oxygen of the CO group of amino acid *n* is hydrogen bonded to the hydrogen of the NH group of amino acid *n+2* and *n+4*, respectively. The conformationally constrained reverse-turns of this invention "mimic" the three-dimensional structure of beta-turns, gamma-turns and beta-bulges, and thus serve as surrogates for such reverse-turn structures present in naturally-occurring proteins and/or peptides.

More specifically, a conformationally constrained beta-turn mimetic of this invention has the following structure I or II:



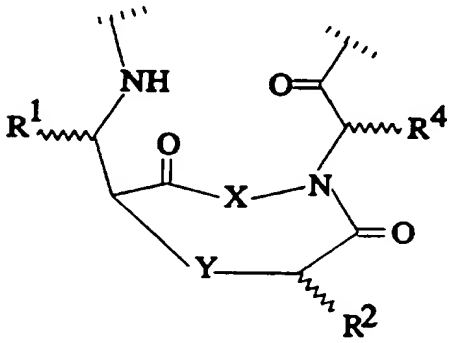
I



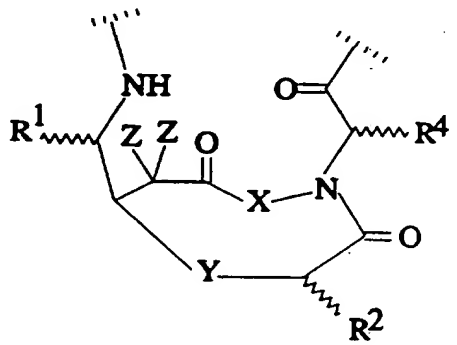
II

wherein R^1, R^2, R^3 and R^4 are amino acid side chain moieties or derivatives thereof, is selected from the chemical moieties identified in Table 1, Y is selected from -CH-, -N(Z)-, -O- and -S-, and Z is -H or $-CH_3$.

A conformationally constrained gamma-turn mimetic of this invention h the following structure III or IV:



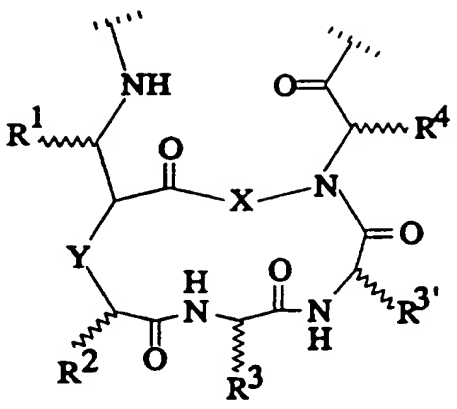
III



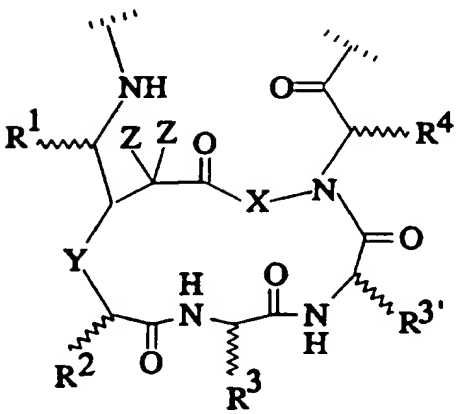
IV

wherein R^1, R^2 and R^4 are amino acid side chain moieties or derivatives thereof, X selected from the chemical moieties identified in Table 1, Y is selected from $-CH_2$ -N(Z)- , -O- and -S-, and Z is -H or $-CH_3$.

A conformationally constrained beta-bulge mimetic of this invention h the following structure V or VI:



V

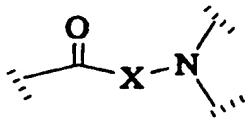


VI

wherein R^1, R^2, R^3, $R^{3'}$ and R^4 are amino acid side chain moieties or derivatives thereof, X is selected from the chemical moieties identified in Table 1, Y is selected from $-CH_2-$, -N(Z)-, -O-, and -S-, and Z is -H or $-CH_3$.

Table 1

"X" Moieties of Structures I through VI



where X =

-NH-

$-NHC(Z)_2CH_2CH_2CH_2-$

$-NH(CH_2)_n-$

$-NH(CH_2)_nCH=CH-$

$-NHC(Z)_2(CH_2)_nNH-$

$-NHC(Z)_2CH=CH(CH_2)_n-$

$-NHC(Z)_2CH=C(Z)(CH_2)_n-$

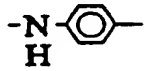
$-NHNHC(Z)_2CH=CH(CH_2)_n-$

$-NHC(Z)_2(CH_2)_nCH=C=N-$

$-NHC(Z)_2(CH_2)_nC\equiv CCH=N-$

$-NHC(Z)_2(CH_2)_nCH=CHCONH-$

$-NHC(Z)_2(CH_2)_nCH=CHCH_2NH-$



$-NHC(Z)_2CH_2-$

$-NHC(Z)_2CH=CHCH_2-$

$-NHC(Z)_2(CH_2)_n-$

$-NH(CH_2)_nC\equiv C-$

$-NHNHC(Z)_2(CH_2)_n-$

$-NHC(Z)_2C\equiv C(CH_2)_n-$

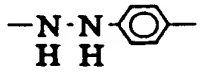
$-NHC(Z)_2CH=CH(CH_2)_nNH-$

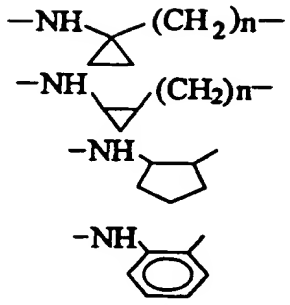
$-NHC(Z)_2(CH_2)_nCH=N-$

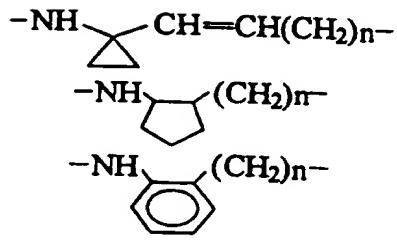
$-NHC(Z)_2(CH_2)_nCH=CHCH=N-$

$-NHC(Z)_2(CH_2)_nCONH-$

$-NHC(Z)_2(CH_2)_nC\equiv CCONH-$

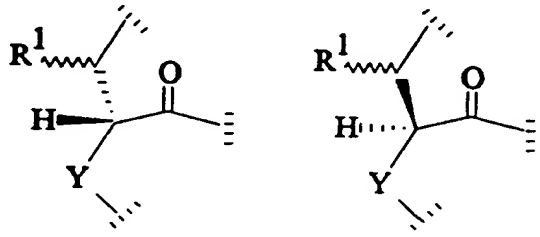






(where n=1-4 and Z = H or CH_3)

As used herein, the designation " ~~~ R" indicates that the amino a side chain moiety (or derivative thereof) may lie either above or below the plane of page. In the case of naturally occurring amino acids (i.e., "L-amino acids"), the R am acid side chain moieties would lie below the plane of the page in structures I through above (i.e., " ····· R"). However, if one or more D-amino acids were employed, corresponding R amino acid side chain moiety would lie above the plane of the page the above structures (i.e., " ▬ R"). In a preferred embodiment, L-amino acids employed to more closely mimic the structure of native protein. The designati " ····· " indicates the remainder of the molecule. In other words, additional chemi moieties are covalently attached to the terminal carbonyl and amine groups of structu I through VI above. This aspect of the invention is addressed in greater detail belo Moreover, it should be understood that the chiral carbon immediately adjacent to t carbon atom having the amino acid side chain moiety R^1 of structures I through VI m be in either the α- or β-position. In other words, this carbon atom may have either the following structures:

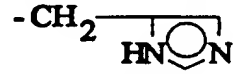
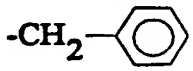
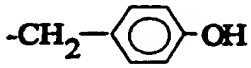
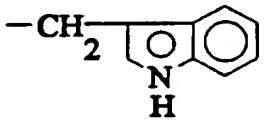
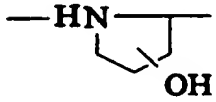


As used herein, the term "an amino acid side chain moiety" represents a amino acid side chain moiety present in naturally occurring proteins, including (but n limited to) the naturally occurring amino acid side chain moieties identified in Table Other naturally occurring amino acid side chain moieties of this invention include (b are not limited to) the side chain moieties of 3,5-dibromotyrosine, 3,5-diiodotyrosin

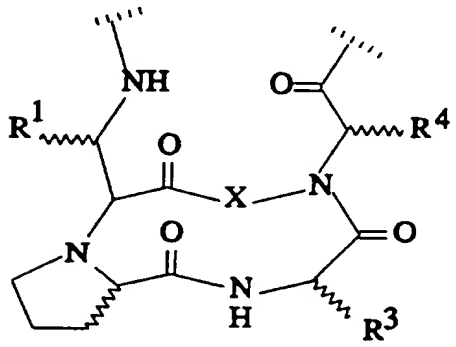
hydroxylysine, γ-carboxyglutamate, phosphotyrosine and phosphoserine. In addition, glycosylated amino acid side chains may also be used in the practice of this invention, including (but not limited to) glycosylated threonine, serine and asparagine.

5

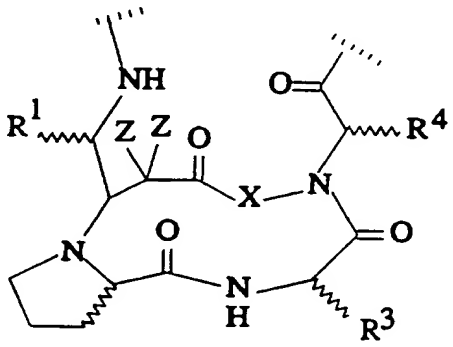
Table 2
Amino Acid Side Chain Moieties

Amino Acid Side Chain Moiety	Amino Acid
-H	Glycine
$-CH_3$	Alanine
$-CH(CH_3)_2$	Valine
$-CH_2CH(CH_3)_2$	Leucine
$-CH(CH_3)CH_2CH_3$	Isoleucine
$-(CH_2)_4NH_3^+$	Lysine
$-(CH_2)_3NHC(NH_2)NH_2^+$	Arginine
	Histidine
$-CH_2COO^-$	Aspartic acid
$-CH_2CH_2COO^-$	Glutamic acid
$-CH_2CONH_2$	Asparagine
$-CH_2CH_2CONH_2$	Glutamine
	Phenylalanine
	Tyrosine
	Tryptophan
$-CH_2SH$	Cysteine
$-CH_2CH_2SCH_3$	Methionine
$-CH_2OH$	Serine
$-CH(OH)CH_3$	Threonine
	Proline
	Hydroxyproline

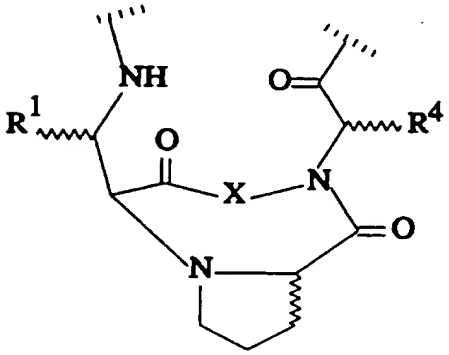
When the amino acid side chain moiety of structures I through VI proline, the five-membered pyrrolidine ring may be a component of the conformation constrained reverse-turn mimetic. In other words, proline may be present at any locati within the conformationally constrained reverse-turn mimetic in place of one or m "-NH-CH(R)-" moieties. For example, inclusion of proline in structures I through above at the "-Y-CH(R^2)-" position (where Y = N) yields the following structures I through VI(a):



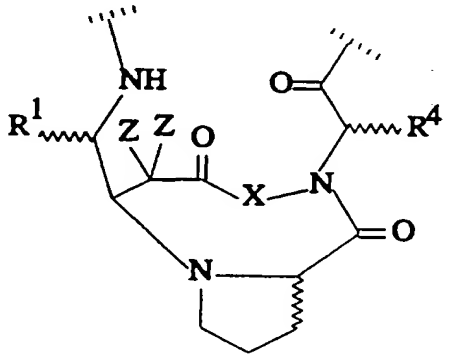
I(a)



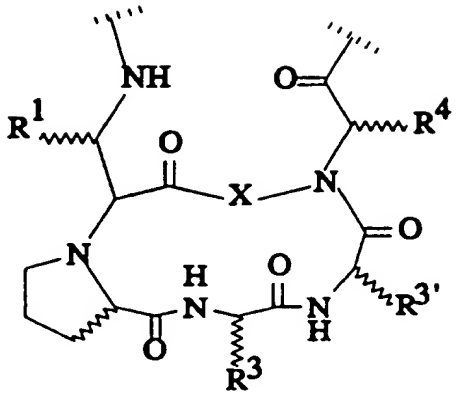
II(a)



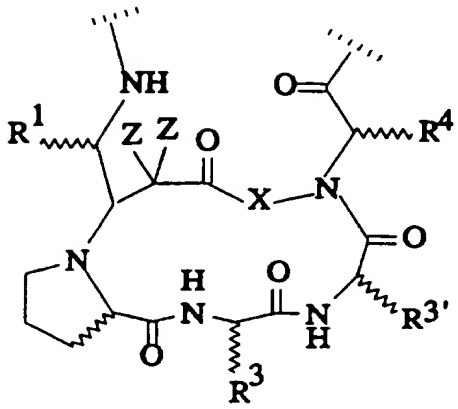
III(a)



IV(a)



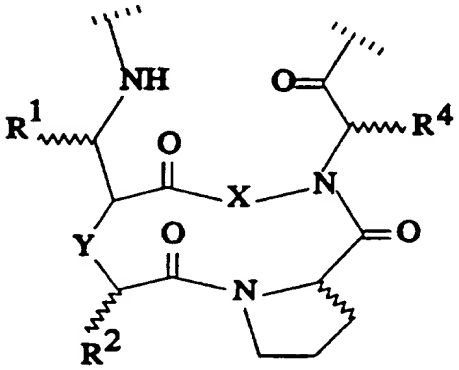
V(a)



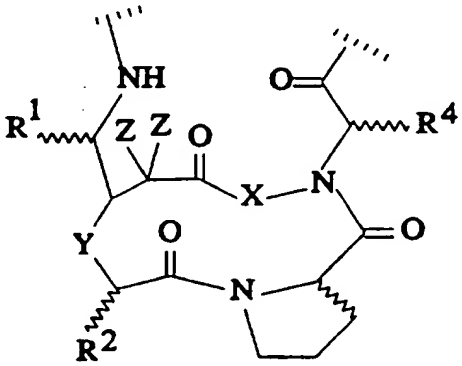
VI(a)

where R^1, R^3, $R^{3'}$, R^4, X and Z are as identified above with regard to structures I through VI.

Alternatively, structures I and II may be modified by inclusion of proline within the conformationally constrained reverse-turn mimetic at the "-NH-CH(R^3)-" position, yielding structures I(b) and II(b) below:

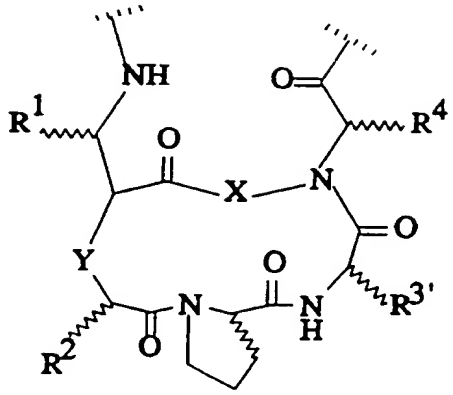


I(b)

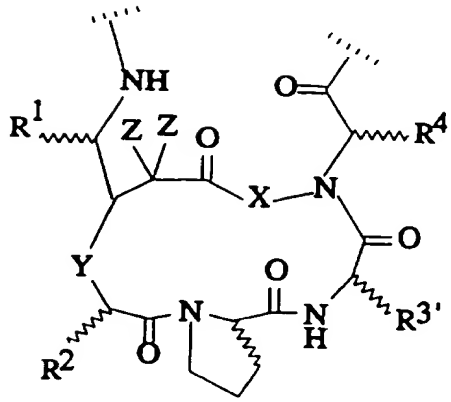


II(b)

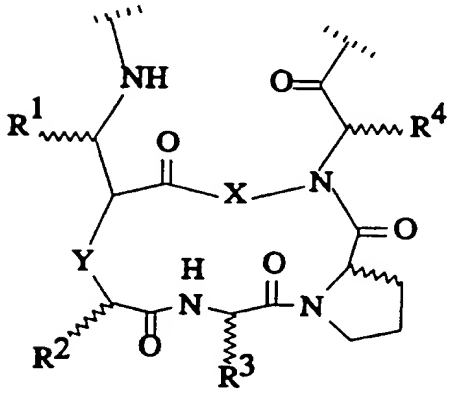
Similarly, structures V and VI may be modified by inclusion of proline within the conformationally constrained reverse-turn mimetic at the "-NH-CH(R^3)-" or "-NH-CH($R^{3'}$)-" positions, yielding structures V(b), VI(b), V(c) and VI(c):



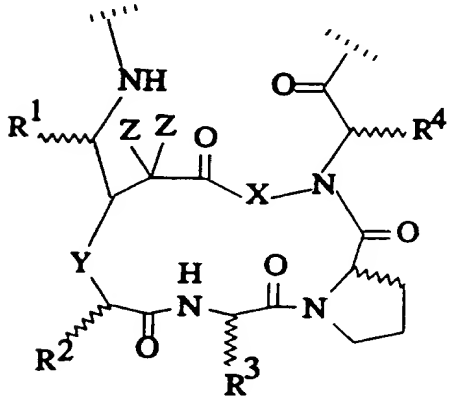
V(b)



VI(b)



V(c)



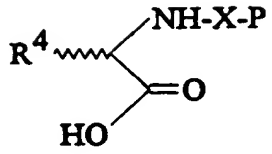
VI(c)

where R^1, R^2, R^3, $R^{3'}$, R^4, X, Y and Z are as identified above with regard to structu I through VI.

Accordingly, general structures I through VI above include structu I(a), II(a), III(a), IV(a), V(a), VI(a), I(b), II(b), V(b), VI(b), V(c) and VI(c) representative examples where the amino acid side chain moiety is proline (or derivative thereof).

The conformationally constrained reverse-turn mimetics of the prese invention are made by utilizing appropriate starting component molecules (hereinafi referred to as "component pieces"). In short, first, second and third component piec are combined (in various combinations) and then cyclized to yield the conformationa constrained reverse-turn mimetics of this invention.

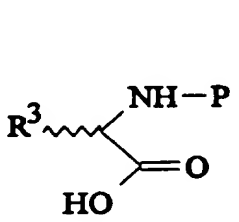
Within the context of this invention, a "first component piece" has t following structure 1:



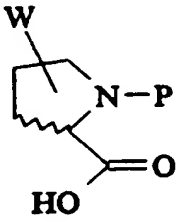
1

wherein R^4 is an amino acid side chain moiety or derivative thereof, X is selected from the chemical moieties identified in Table 1, and P is a protective group suitable for use in peptide synthesis.

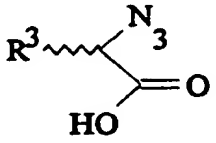
A "second component piece" of this invention is selected from the following structures 2(a), 2(b) and 2(c):



2(a)



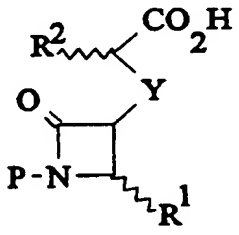
2(b)



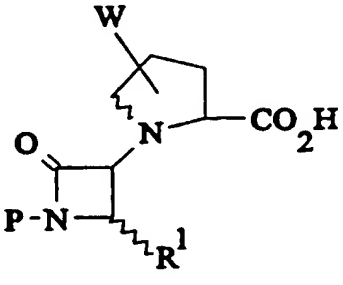
2(c)

wherein R^3 is an amino acid side chain moiety or derivative thereof, W is -H, -OH alkyl, aryl, -O-alkyl, -O-aryl, -S-alkyl or -S-aryl, and P is a protective group suitable for use in peptide synthesis.

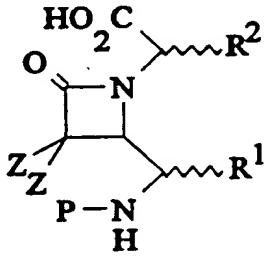
A "third component piece" of this invention is selected from the following structures 3(a), 3(b) and 3(c):



3(a)

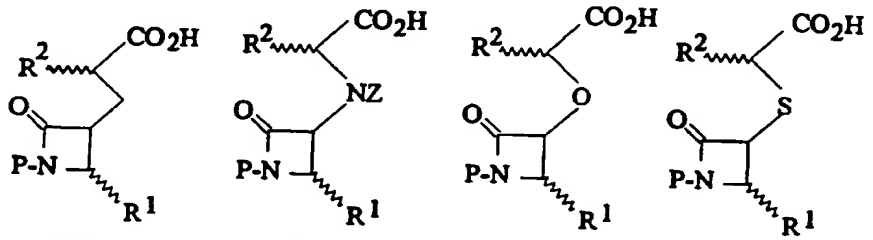


3(b)



3(c)

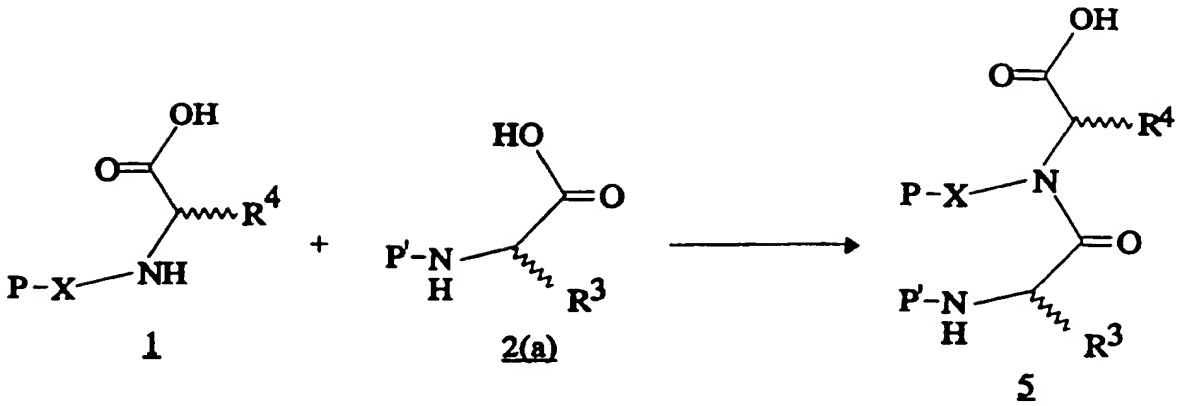
where R^1 and R^2 are amino acid side chain moieties or derivatives thereof, Y is -CH -NZ-, -O- or -S-, Z is H or methyl, W is -H, -OH, alkyl, aryl, -O-alkyl, -O-aryl, -S-alk or -S-aryl, and P is a protective group suitable for use in peptide synthesis. Thus, wh Y of structure 3(a) is $-CH_2-$, -NZ-, -O- or -S-, the following third modular compon pieces 3(a1), 3(a2), 3(a3) and 3(a4), respectively, are obtained:



3(a1) 3(a2) 3(a3) 3(a4)

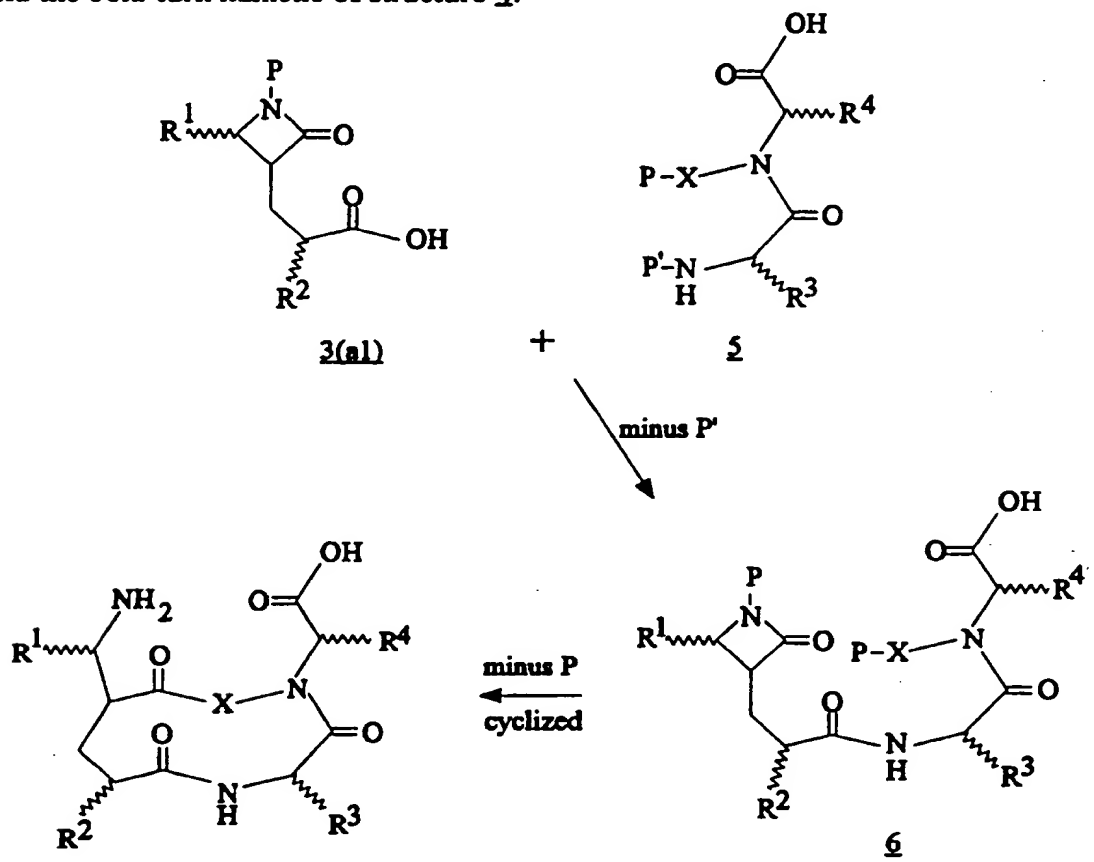
More specifically, conformationally constrained beta-turn mimetics of th invention (see structures I and II above) are synthesized by reacting a first compone piece with a second component piece to yield a combined first-second intermediat followed by reacting the first-second intermediate with a third component piece to yiel a combined first-second-third intermediate, and then cyclizing the resulting first-seconc third intermediate to yield the conformationally constrained beta-turn mimeti Conformationally constrained gamma-turn mimetics (*see* structures III and IV above are synthesized in the same manner, except that the second component piece is omitte (i.e., a first-third intermediate is cyclized). Similarly, conformationally constrained beta bulges (*see* structures V and VI above) are synthesized by use of two second componen pieces (i.e., a first-second-second-third intermediate is cyclized).

For example, the general synthesis of a conformationally constraine beta-turn mimetic having structure 4 below may be synthesized by the following technique. A first modular component piece 1 is combined with a second modula component piece 2(a) to yield a first-second intermediate 5 as illustrated below (it should be recognized that following reaction scheme is presented for illustration purposes, and that the carboxylic acid moiety of structure 1 should be suitably protected by, for example, esterification, by formation of an amide bond, or by attachment to a solid support):



1 2(a) 5

A third modular component piece 3(a1) is then combined with the first-second intermediate 5 to give a pre-cyclized beta-turn mimetic 6, which in turn is cyclized to yield the beta-turn mimetic of structure 4:



3(a1) 5 6 4

All conformationally constrained reverse-turn mimetics of this invention may be synthesized by appropriate choice of the various component pieces by the general procedure outlined above. For example, R group variations to structures I

through VI may be made by use of appropriate first, second and third component pie possessing the desired R group. Similarly, conformationally constrained gamma-t mimetics may be synthesized by linking a first component piece with a third compor piece (omitting the second component piece), followed by cyclization. Alternatively first component piece may be combined with a second component piece, followed combination with yet another second component piece to yield a first-second-sec intermediate. This intermediate may then be combined with a third component piece cyclized to yield the corresponding conformationally constrained beta-bulge mime Additional disclosure directed to the synthesis of the conformationally constrai reverse-turn mimetics of this invention is set forth in Examples 1-3 below.

Synthesis of the peptide mimetics of the library of the present invent may be accomplished using known peptide synthesis techniques, in combination with first, second and third component pieces of this invention. More specifically, any ami acid sequence may be added to the N-terminal and/or C-terminal of the conformation constrained reverse-turn mimetic. To this end, the mimetics may be synthesized or solid support (such as PAM resin) by known techniques (*see, e.g.*, John M. Stewart a Janis D. Young, *Solid Phase Peptide Synthesis*, 1984, Pierce Chemical Com Rockford, Illinois).

In addition, a combination of both solution and solid phase synthe techniques may be utilized to synthesize the peptide mimetics of this invention. F example, a solid support may be utilized to synthesize the linear peptide sequence up the point that the conformationally constrained reverse-turn is added to the sequence. suitable conformationally constrained reverse-turn mimetic which has been previou synthesized by solution synthesis techniques may then be added as the next "amino aci to the solid phase synthesis (i.e., the conformationally constrained reverse-turn mimet which has both an N-terminus and a C-terminus, may be utilized as the next amino ac to be added to the linear peptide). Upon incorporation of the conformational constrained reverse-turn mimetic into the sequence, additional amino acids may then l added to complete the peptide vaccine bound to the solid support. Alternatively, tl linear N-terminus and C-terminus protected peptide sequences may be synthesized on solid support, removed from the support, and then coupled to the conformational constrained reverse-turn mimetic in solution using known solution coupling techniques.

In another aspect of this invention, methods for constructing the libraries a disclosed. Traditional combinatorial chemistry techniques (*see, e.g.*, Gallop et al., *Med. Chem.* 37:1233-1251, 1994) permit a vast number of compounds to be rapid prepared by the sequential combination of reagents to a basic molecular scaffol

Combinatorial techniques have been used to construct peptide libraries derived from the naturally occurring amino acids. For example, by taking 20 mixtures of 20 suitably protected and different amino acids and coupling each with one of the 20 amino acids, a library of 400 (i.e., 20^2) dipeptides is created. Repeating the procedure seven times results in the preparation of a peptide library comprised of about 26 billion (i.e., 20^8) octapeptides.

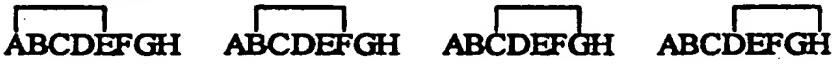
The peptide library described above results in a traditional combinatorial library derived from naturally occurring amino acids. Traditional combinatorial libraries consist of members differing only in the sequence in which its constituents are combined. For example, a library of octapeptides consists of members including ABCDEFGH, ACBDEFGH, ABDCEFGH, etc., where A-H represent amino acids. In the practice of the present invention, the library is composed of conformationally constrained reverse-turn mimetics. That is, for any given peptide sequence, a multitude of reverse-turns are possible. Accordingly, the libraries of the present invention may be significantly larger and more diverse than a library constructed solely by sequence variation of a linear peptide. Such reverse-turn mimetic libraries provide not only compounds of fixed sequence, but within each fixed sequence, provide a family of mimetics of various fixed conformations.

To illustrate this point, a representative library of reverse-turn mimetics for the peptide ABCDEFGH are presented schematically below.

Beta-Turns:

Gamma-Turns:

Beta-Bulges:



The above library generally comprises 5 beta-turn mimetics, 6 gamma-turn mimetics and 4 beta-bulge mimetics. Moreover, for each one of the above representations, additional reverse-turn mimetics may be constructed. As disclosed above, the conformation of the reverse-turn mimetics are constrained by cyclizing the first and third component pieces

through covalent coupling of linking group X of the first component piece with carbonyl of the azetidinone group of the third component piece (*see* Example 3). T presence of additional component pieces between the first and third component pie determines the nature of the reverse-turn mimetic: cyclization of first-second-third, fi third, and first-second-second-third component pieces yield beta-turn, gamma-turn, a beta-bulge mimetics, respectively. Accordingly, in addition to the multiplicity of rever turns possible for a given sequence, variation of linking group X (*see* Table 1) provi even greater diversity of the mimetic library.

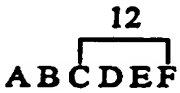
The libraries of the present invention thus differ from existing combinato libraries of linear peptides. For example, in most hexapeptide libraries (i.e., ABCDE each position is randomized to afford libraries of 10^6 to 10^7 members (i. A(20)B(20)C(20)D(20)E(20)F(20) = 6.4 X 10^7 members). Although it is conceptua possible to generate libraries of this type for reverse-turn mimetics, realistically it is r attractive for several reasons. First, the reverse-turn library is necessarily larger becat of the number of possible linker groups X and, therefore, there is a smaller amount any one component in the library, thus making any single component harder to fi (Medynski, *Biotechnology* 12:709-710, 1994). Second, a large number of unnatu building blocks need to be initially synthesized. Third, the libraries of this invention a not suitable for direct sequencing. Linear peptide libraries can be sequenced usi peptide microsequencing techniques, which can provide sequence information on as lit as picomolar levels of material (*see, e.g.*, Lam et al., *Nature* 354:82-84, 199 However, in order for this technique to work, one must be able to cleave the individu amino acids in the peptide. The reverse turn mimetics of this invention do not have tl capabililty within the conformationally constrained ring, which thus precludes this ty of sequencing.

Accordingly, in the practice of this invention, the libraries are based on a knov linear peptide sequence as the starting point. The peptide sequence may be derived fro a naturally occurring peptide or protein, or may be generated via a biopolymer libra such as a synthetic peptide or phage display. Once the peptide sequence is chosen, t libraries of the present invention are preferably constructed in two phases: (determination of the best template, and (2) optimization of the interaction of the si chains of the selected template. Both of these phases involve evaluation of t interaction of the individual library members with a particular biological target of intere as discussed below.

The determination of the best template entails evaluation of various possit reverse-turns for the particular sequence chosen. In other words, the first phase search

out the shape of the mimetic for presentation of the sequence information. By way of example, assuming a hexapeptide of the sequence ABCDEF as the peptide of interest, a library of the type shown below is first constructed.

The above represents a nested set of beta-turn, gamma-turn, and beta-bulge turn mimetics of different sizes and conformations based on the primary sequence ABCDEF. The above library consists of three beta-turns, with each turn containing a different linker group X. For example, if three different linker groups are used, beta-turn mimetics containing 10-, 12-, and 14-membered rings may be prepared (*see* Example 3). Similarly, the above library also consists of four gamma-turn mimetics, and by appropriate selection of X, gamma-turn mimetics containing 7-, 9-, and 11-membered rings may be prepared. The above library further consists of two beta-bulge mimetics which, using three different X linker groups, results in beta-bulge mimetics containing 13-, 15-, and 17-membered rings. With the above library thus constructed, the selection of the best template is performed by contacting the library members with the target of interest in a suitable assay. For example, the best template (as determined by the positive interactions or "hits" of the assay) may be a beta-turn containing a 12-membered ring such as shown below.



Once the best template is selected, the side chains of the template are then optimized. In the side chain optimization step, the template is maintained and the various residues (A through F) are randomized. The randomization involves the synthesis of additional compounds in which other side chains (other amino acid derived component pieces) are substituted. Both turn residues (C-F) and adjacent residues (A-B) may be randomized. The randomization may be carried out in a single screen, or alternatively, the randomization may be separated into two or more rounds of screening. As with template selection, the determination of the optimal side chains is achieved by screening using an appropriate assay. The results of the screening assay may provide a beta-turn containing a 12-membered ring with a sequence as shown below.

12

A B G D H F

The preparation of a library by the above method is described in Example 4.

In a further aspect of this invention, methods for the screening the libraries f bioactivity and isolating bioactive library members are disclosed. The libraries of t present invention may be screened for bioactivity by a variety of techniques a methods. Generally, the screening assay may be performed by (1) contacting a libra with a biological target of interest, such as a receptor, and allowing binding to occ between the mimetics of the library and the target, and (2) detecting the binding event b an appropriate assay, such as by the colorimetric assay disclosed by Lam et al. (*Natu* **354**:82-84, 1991) or Griminski et al. (*Biotechnology*, **12**:1008-1011, 1994) (both which are incorporated herein by reference). In a preferred embodiment, the libra members are in solution and the target is immobilized on a solid phase. Alternativel the library may be immobilized on a solid phase and may be probed by contacting it wi the target in solution.

As discussed above, when the library of this invention is spatially addressable ar a positive interaction "hit" is achieved, the structure of the bioactive mimetic will b known, or known to be of a limited number of structures. In the case of nonspatial addressable libraries of this invention, depending upon the particular embodiment of th screening bioassay, certain characteristics and properties may be imposed upon th reverse-turn mimetic to facilitate the recognition of bioactivity in a screening assay ar also to permit the isolation and identification of the mimetic. For example, a recepto bound bioactive mimetic may be collected through a binding interaction with a soli phase immobilized ligand-binding protein, such as avidin immobilized on a magneti bead. In such a screening assay, to effect binding of the receptor-bound mimetic to th immobilized ligand-binding protein, the mimetic need possess a suitable ligand. In th above mentioned example where the immobilized ligand binding protein is avidin, th mimetic ligand is preferably biotin.

In the above example, once the biotin of the receptor-bound mimetic has bee bound to the avidin immobilized on magnetic beads, the bioactive mimetic may be readil removed from the immobilized receptor by applying a magnetic field. For example contacting the magnetic beads with a magnet is effective in separating the mimetic fror the receptor. To identify the bioactive mimetic, the mimetic is then released from th magnetic solid phase by, for example, cleavage. Cleavage of the mimetic from th magnetic bead may occur through either chemical or enzymatic means. In either case

the mimetic should possess a suitable cleavable linkage. In a preferred embodiment, the mimetic is cleaved from the magnetic bead by enzymatic means and the cleavable linkage is a peptide sequence susceptible to enzymatic cleavage. For example, the enzyme thrombin effectively cleaves the sequence GRG (glycine-arginine-glycine). Accordingly, thrombin treatment of a magnetically bead bound mimetic possessing a cleavable link GRG results in cleavage of the GRG sequence and release of the bioactive mimetic. Additional strategies for cleavable linkers are disclosed by Patek et al., *Int. J. Peptide Protein Res.* 42:97-117, 1993 (incorporated herein by reference).

The conformationally-constrained reverse-turn mimetics of the present invention may thus incorporate a cleavable linker to facilitate release of a bioactive mimetic from the mimetic screening format prior to mimetic identification. Preferred linkers are amino acid sequences capable or prone to proteolytic breakdown. The cleavable linker of this invention is preferably located between the conformationally constrained reverse-turn mimetic and the ligand (such as biotin or the trypsin-like serine protease Factor X).

A further cleavable linker of this invention contains a recognition site (i.e., cleavage site) for the aspartic protease cathepsin D. Other cleavable linkers of this invention include, but are not limited to, the amino acid sequences which are cleavable by cathepsin B or by cathepsin E. Cathepsin B is a cystein proteinase which cleaves sequences having a basic amino acid (such as arginine or lysine) at the P_2 and P_3 positions, a small hydrophobic moiety at P_1, and an aromatic hydrophobic moiety at P_1' and P_4 (*see* Matsueda et al., *The Chemical Society of Japan - Chemistry Letters* pp. 1857-1860, 1988; Matsunaga et al., *FEBS* 324:325-330, 1993). Similarly, cathepsin E is an endosomal aspartic proteinase present in both B and T cells, but not in peritoneal macrophages. The active cleft of this enzyme is capable of accommodating as many as nine residues, with a distinct preference for cleavage to occur between hydrophobic residues occupying the P_1-P_1' sites (*e.g.* Bennett et al., *Eur. J. Immunol.* 22:1519-1524, 1992).

Therefore, for the above described screening method, the mimetic includes both a ligand (such as biotin for effective collection from the receptor), and a cleavable linkage (such as a peptide sequence susceptible to enzymatic cleavage). Such mimetics may be prepared by traditional chemistries that are complementary to and may be directly incorporated into the solid or liquid phase synthesis of the mimetics described above. To that end, the incorporation of biotin and cleavable linkages are preferably made on the C terminus of the mimetic. The mimetic may be schematically represented by the following representation:

N-terminus - reverse-turn mimetic - C terminus - cleavable link - ligand

The above mimetics may be synthesized by the same methods which yield t reverse-turn mimetics themselves. Briefly, in order to incorporate a ligand such as bio into the mimetic, a lysine residue is incorporated into the mimetic as the first resid The nucleophilic epsilon amino group of the lysine side chain serves as the reactive s for the coupling of a ligand such as biotin. Treatment of the lysine residue with reactive form of biotin such as the N-hydroxysuccinimde of biotin results in coupling biotin to the mimetic. The peptide mimetic synthesis may then be continued by t addition amino acid residues suitable to make up the cleavage site. For instance, mentioned above, the residue glycine, arginine, and glycine may be coupled to provi such a cleavable link. Once both the biotin and the cleavable link are in place, pepti mimetic synthesis follows as described above and in Examples 1-3.

In yet another aspect of the invention, methods for the identification bioactive mimetics are disclosed. As described above, the bioactive mimetic may released by treatment with a suitable enzyme. The result is a solution containi individual bioactive mimetics and the cleaving enzyme. The bioactive mimetics may separated from the cleaving enzyme by traditional techniques such as size exclusi filtration to provide a solution of bioactive mimetics suitable for analysis. In a preferr embodiment, the solution of bioactive mimetics is analyzed by the combination of liqu chromatography and mass spectrometry (LC-MS). When more than a single bioacti mimetic is collected from the screening assay of the mimetic library, liqu chromatographic analysis provides both a means for separation and quantification of t bioactive mimetics. The chromatogram of such a mixture provides a binding profile the mimetics. Elution of the liquid chromatogram into the mass spectrometer provid unambiguous identification of the bioactive mimetics.

The following examples are offered by way of illustration, not limitation

EXAMPLES

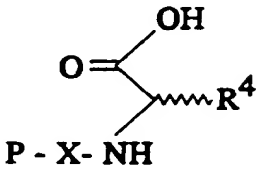
Example 1

Synthesis of Component Pieces

This example presents the synthesis of the component pieces which combine t form the conformationally constrained reverse-turn mimetics of the present invention.

A. Synthesis of First Component Pieces

The first component piece of this invention has the following structure 1:



1

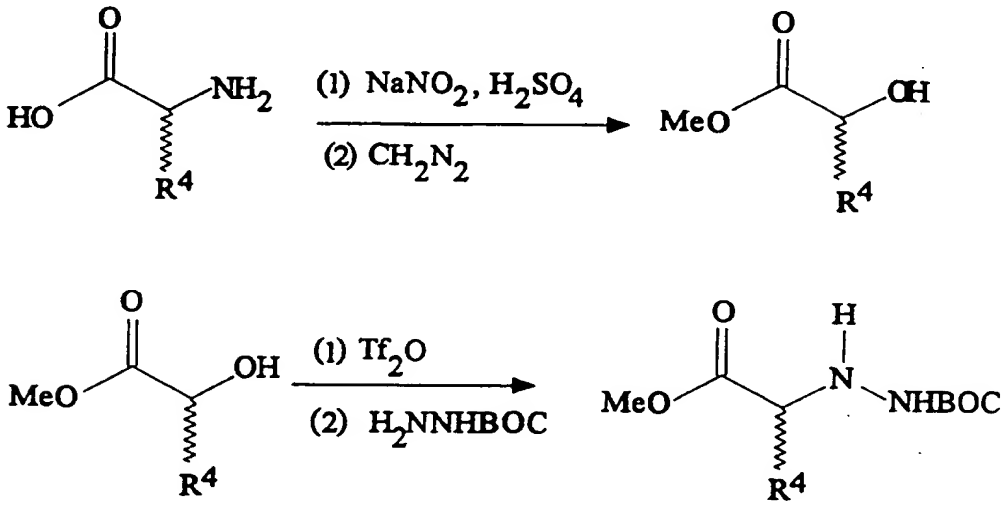
where R^4 is an amino acid side chain moiety or derivative thereof, X is a chemical moiety selected from the moieties identified in Table 1, and P is an amino protective group suitable for use in peptide synthesis. The "X" moiety establishes the bridge between the first and third component pieces which defines the ring size of the conformationally constrained reverse-turn mimetic. The X moiety may be variable in length and flexibility, and thus effects the three-dimensional structure of the conformationally constrained reverse-turn mimetic.

The first component piece may be an N-protected hydrazine where the moiety X is a single nitrogen atom. Upon cyclization to the reverse turn mimetic, such a first component piece forms a hydrazide link with the carbonyl group of the third component piece. Alternatively, the first component piece may be an N-protected amine which upon cyclization forms an amide link. The synthesis of both general types of first compound pieces is set forth below.

(1) Synthesis of N-Protected Hydrazines (X=NH)

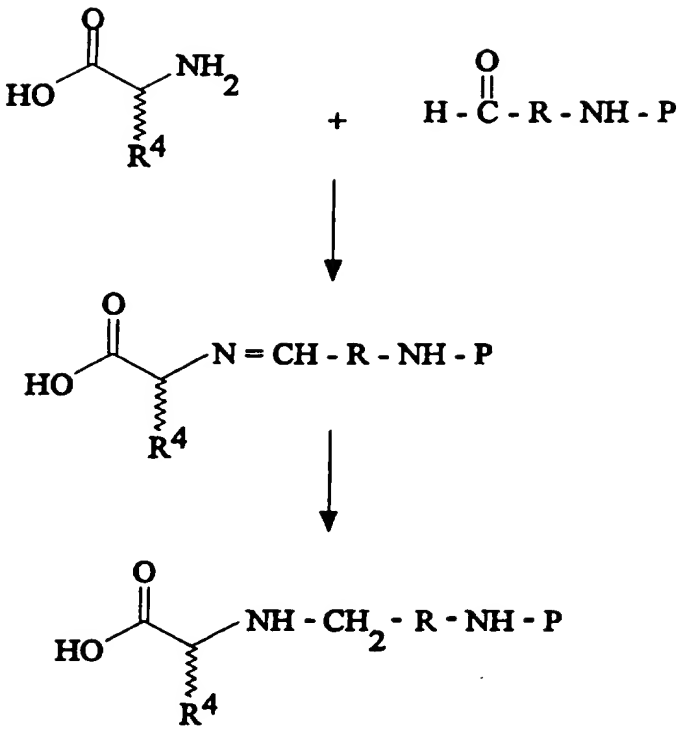
N-protected hydrazines for use as the first component piece may be made from their corresponding amino acids according to the procedures of Hoffman and Kim (*Tet. Lett.* 31:2953, 1990) and Vidal et al. (*J. Org. Chem.* 58:4791-4793, 1993). Briefly, an amino acid may be converted to the corresponding α-hydroxy methyl ester by reaction with sodium nitrite in aqueous sulfuric acid followed by treatment with diazomethane. The α-hydroxy group is displaced with mono-t-butyloxycarbonyl hydrazine after its conversion to trifluoromethanesulfonate with trifluoromethanesulfonic anhydride (with overall inversion of the amino acid configuration about the chiral carbon atom). The result is a first component N-protected hydrazine, a first component piece represented by formula 1 where the carboxylic acid group is protected as a methyl ester, X is NH, and P

is the amino protective group, t-butyloxycarbonyl (BOC). The conversion of an ar acid to a representative first component N-protected hydrazine is shown below.



(2) Synthesis of First Component N-Protected Amines (X=NHRCH$_2$)

Reductive amination. A variety of first component pieces may be prepared fr suitable aldehydes by a facile reductive amination process, as described by Gribble a Nutatitis (*Org. Prep. Proced. Int.* 17:317, 1985) or Sasaki and Coy (*Peptides* 8:1 1987). In this method, reaction of the amino group of an amino acid with the carbo group of an aldehyde results in the formation of an imine which may be subsequer reduced with an appropriate hydride reducing agent to provide a C-N link. To prod such a first component piece of the present invention, the requisite aldehyde bears a protected amino group. The synthesis of first component N-protected amines by gene method of reductive amination is presented schematically below (with overall retent of the amino acid configuration about the chiral carbon atom):

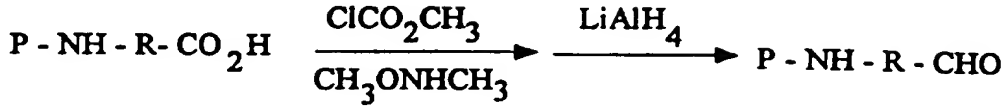


Alternatively, synthesis of the above N-protected amines may be accomplished on solid support by substituting the HOOC-CH(R^4)-NH_2 moiety with P—OOC—CH(R^4)—NH_2 to yield P—OOC—CH(R^4)—$NHCH_2$RNHP.

The N-protected amino aldehyde shown above may be represented with the general formula, P-NH-R-CHO, where P is an amino protecting group and R is a chemical moiety (for example, -CH_2-, -$C(CH_3)_2$-, -CH=$CHC(CH_3)_2$-, or -$CH_2CH_2C(CH_3)_2$-) which links the aldehyde carbonyl with the N-protected amino group. The product of the reductive amination shown above yields the first component piece of structure 1 when X is NH-R-CH_2.

Synthesis of aldehydes from amino acids. The preparation of first component pieces with variable X by the reductive amination method requires the synthesis of suitable aldehydes, i.e., aldehydes which bear N-protected amino groups. Such suitable aldehydes may be directly prepared from their corresponding amino acids by a two step procedure as described by Goel et al. (*Org. Syn.* 67:69, 1988). In a typical reaction sequence, an N-protected amino acid is first converted to a mixed anhydride by treatment with methyl chloroformate and treatment with N,O-dimethylhydroxylamine provides the corresponding carboxamide, which is followed by reduction to the aldehyde with lithium aluminum hydride. The conversion of an N-protected amino acid to its

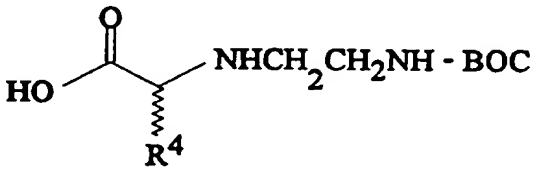
corresponding N-protected aldehyde, as described above, is illustrated schematically the following reaction:



where P and R are as described above. The synthesis of suitable N-amino protec aldehydes is presented as sections (a)-(d) below.

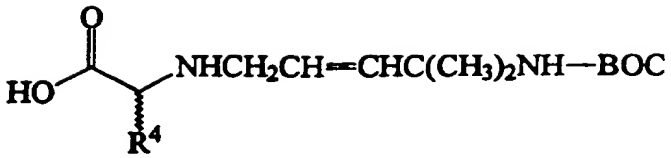
(a) First Component N-Protected Amines (X=$NHCH_2CH_2$)

In this example, the aldehyde is an N-protected 2-aminoethanol and may prepared from its corresponding N-protected amino acid, glycine, according to process of Goel et al. as described in section (2) above. N-BOC-glycine is commerci available from a variety of sources and is suitable for conversion to its aldehyde, BC $NH\text{-}CH_2\text{-}CHO$. Treatment of the resulting aldehyde, N-BOC-aminoacetaldehyde, w an amino acid under the reductive amination conditions as described in (2) above yie the first component N-protected amine shown below, which is the first component pi of general formula 1 when X is $-NHCH_2CH_2-$ and P is the BOC protecting group:



(b) First Component N-Protected Amines (X=$NHC(CH_3)_2CH_2$)

In this example, the aldehyde is an N-protected 2-amino-2-methylpropanal, a may be prepared from its corresponding N-protected amino acid, 2-methylalani according to the process of Goel et al. as described in section (2) above. N-BOC- methylalanine is commercially available from a variety of sources and is suitable conversion to its aldehyde, $BOC\text{-}NH\text{-}C(CH_3)_2\text{-}CHO$. Treatment of the resulti aldehyde, N-BOC-2-amino-2-methylpropanal, with an amino acid under the reducti amination conditions as described in section (2) above yields the first component protected amine shown below which satisfies the first component piece of gene formula 1 where X is $NHC(CH_3)_2CH_2$.



(c) First Component N-Protected Amines ($X=NHC(CH_3)_2CH=CHCH_2$)

Extension of the length and adjustment of the flexibility of linker X may be achieved by homologation of the aldehydes described above. For example, the homologation of the aldehyde intermediate in section (2)(b) above by Wittig reaction provides a vinylogous analog as described in House and Rasmusson (*J. Org. Chem.* **26**:4278, 1961).

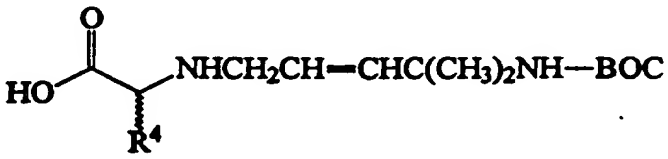
Briefly, treatment of N-BOC-2-amino-2-methylpropanal, from section (2)(b) above, with methyl (triphenylphosphoranylidene) acetate results in the extension of the carbon chain of the aldehyde by two carbons. The reaction is represented schematically as follows.

$$BOC\text{-}NH\text{-}C(CH_3)_2\text{-}CHO + Ph_3P{=}CH\text{-}CO_2CH_3 \longrightarrow BOC\text{-}NH\text{-}C(CH_3)_2\text{-}CH{=}CH\text{-}CO_2CH_3$$

The ester product above may then be hydrolyzed under basic conditions to yield the corresponding N-BOC amino carboxylic acid, which may then be directly converted to the aldehyde by the reductive method of Goel et al. as described above in section (2). Conversion of the carboxylic acid derived from the above ester to the corresponding aldehyde is illustrated below.

$$BOC\text{-}NH\text{-}C(CH_3)_2\text{-}CH{=}CH\text{-}CO_2H \longrightarrow BOC\text{-}NH\text{-}C(CH_3)_2\text{-}CH{=}CH\text{-}CHO$$

Treatment of the vinylogous aldehyde with an amino acid under the reductive amination conditions as described in section (2) above yields the first component N-protected amine shown below, which satisfies the first component piece general formula 1 when X is $NHC(CH_3)_2CH=CHCH_2$:

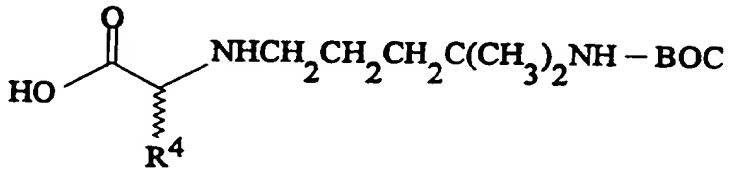


(d) First Component N-Protected Amines
(X=$NHC(CH_3)_2CH_2CH_2CH_2$)

The X moiety of section (2)(c) above contains a vinyl group which imparts sc rigidity to the linker moiety. A more flexible linker may be prepared by hydrogenatio the N-protected vinylogous aldehyde described above. Hydrogenation to the vinylog aldehyde produces the saturated analog shown below.

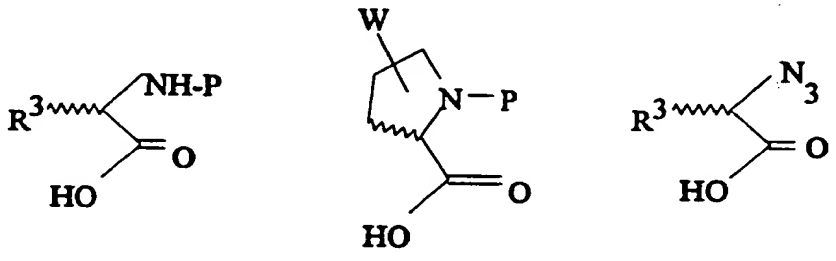
$$BOC\text{-}NH\text{-}C(CH_3)_2\text{-}CH{=}CH\text{-}CHO \longrightarrow BOC\text{-}NH\text{-}C(CH_3)_2\text{-}CH_2CH_2\text{-}CHO$$

Treatment of the saturated aldehyde with an amino acid under the reduct amination conditions as described in section (2) above yields the first component protected amine shown below, which satisfies the first component piece general form 1 when X is $NHC(CH_3)_2CH_2CH_2CH_2$:



B. Synthesis of Second Component Pieces

The second component pieces of this invention may have any one of t following structures 2a, 2b or 2c:

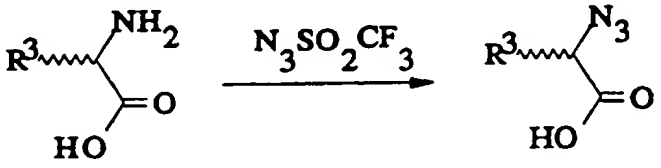


2a 2b 2c

where R^3 is an amino acid side chain moiety or derivative thereof, W is as defin previously, and P is an amino protective group suitable for use in peptide synthesi Structure 2a is a generalized representation of an N-protected amino acid and structu 2b represents an N-protected proline derivative. Suitable second component pieces, 2 and 2b, are commercially available. For example, FMOC amino acids are available fro

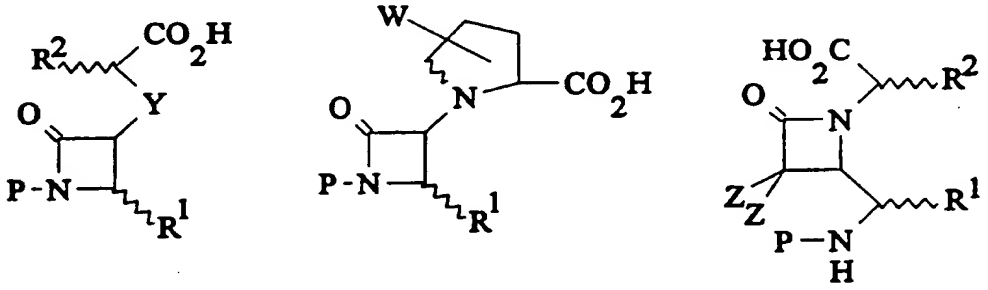
a variety of sources. Alternatively, these or other N-protected amino acids may be readily prepared by standard organic synthetic techniques.

Second component piece 2c is an azido derivative of an amino acid. In this amino acid derivative, the α-amino group has been substituted with an azido group. The azido derivative of an amino acid may be prepared by the following reaction (Zaloom et al., *J. Org. Chem.* 46:5173-5176, 1981):



C. Synthesis of Third Component Pieces

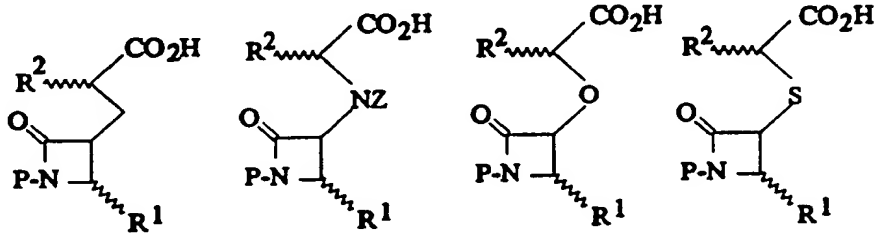
The third component piece may have any one of the following structures:



3(a) 3(b) 3(c)

where R^1 and R^2 are an amino acid side chain moieties or derivatives thereof, Y is $-CH_2-$, -NZ-, -O- or -S-, Z is H or $-CH_3$, W is as defined previously, and P is an amino protective group suitable for use in peptide synthesis. Preferred protective groups include t-butyl dimethylsilyl (TBDMS), BOC, FMOC and Alloc (i.e., allyloxycarbonyl).

More specifically, third component piece 3(a) may have any one of the following structures:

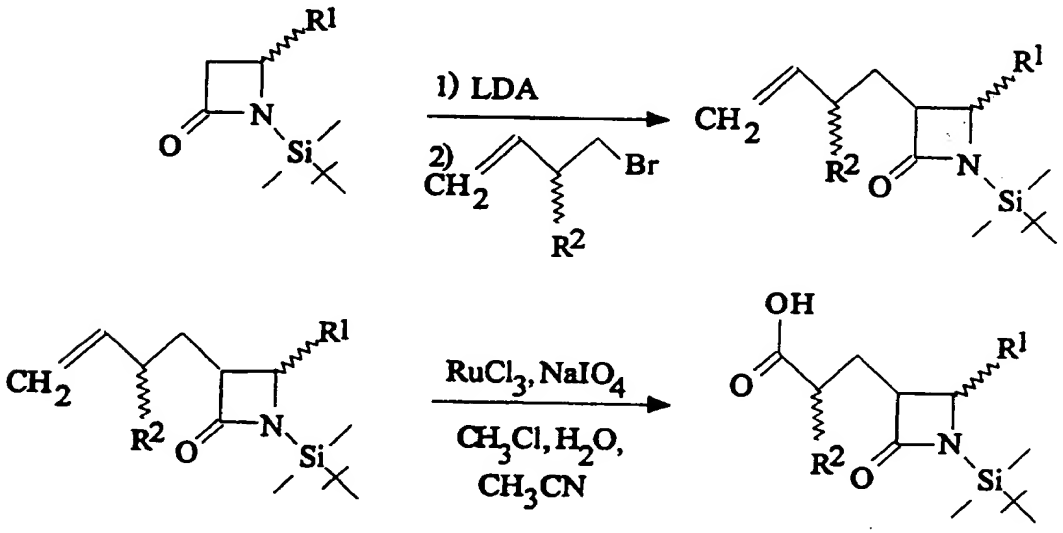


3(a1) 3(a2) 3(a3) 3(a4)

The third component pieces of the present invention are β-propiola derivatives, also commonly known as 2-azetidinone derivatives. Third component pi 3a are analogous azetidinones which differ in the nature of the atom connecting azetidinone ring with the carboxylic acid portion of the molecule. The atom connec the two portions of these third component pieces are C, N, O and S for 3(a1), 3(3(a3) and 3(a4), respectively. Third component piece 3b is a derivative of 3a wherein R^2 is proline. Third component piece 3c is a 2-substituted azetidinone in w the azetidinone nitrogen is also the α-amino group of an α-amino acid. Represents syntheses of these third component pieces 3a-3c follows.

(1) Synthesis of Third Component Piece 3(a1)

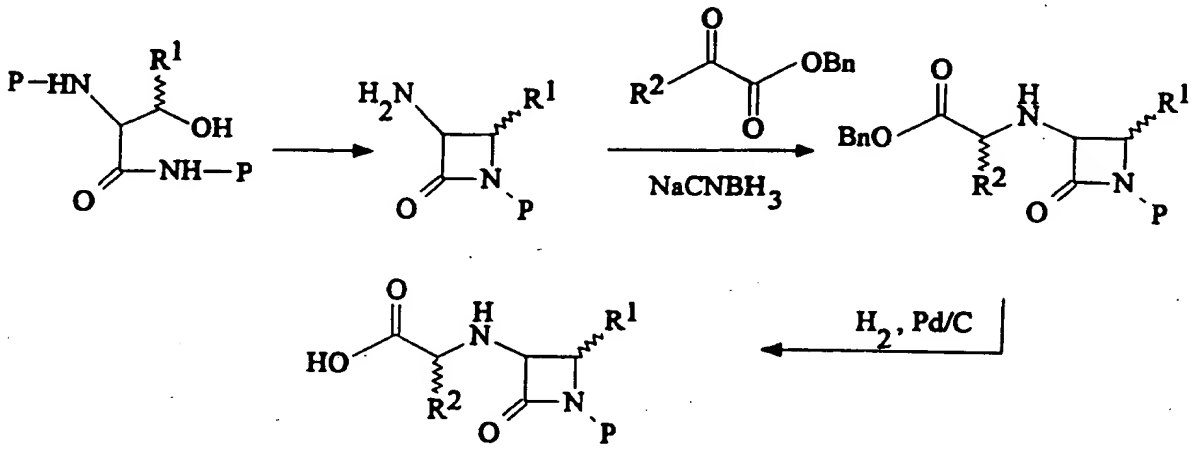
Third component piece 3(a1) may be prepared by the method as gener described in Williams et al., *J. Amer. Chem. Soc.* 111:1073, 1989. The synthesis 3(a1) is presented schematically below.



Briefly, the anion of an N-TBDMS-2-azetidinone generated by treatment with lithi diisopropylamide is alkylated with a 4-bromobutenyl derivative to yield corresponding 4-butenyl azetidinone. Oxidative cleavage of the terminal alkene w ruthenium tetroxide provides third component piece 3(a1), where P is the TBDM protective group.

(2) Synthesis of Third Component Piece 3(a2)

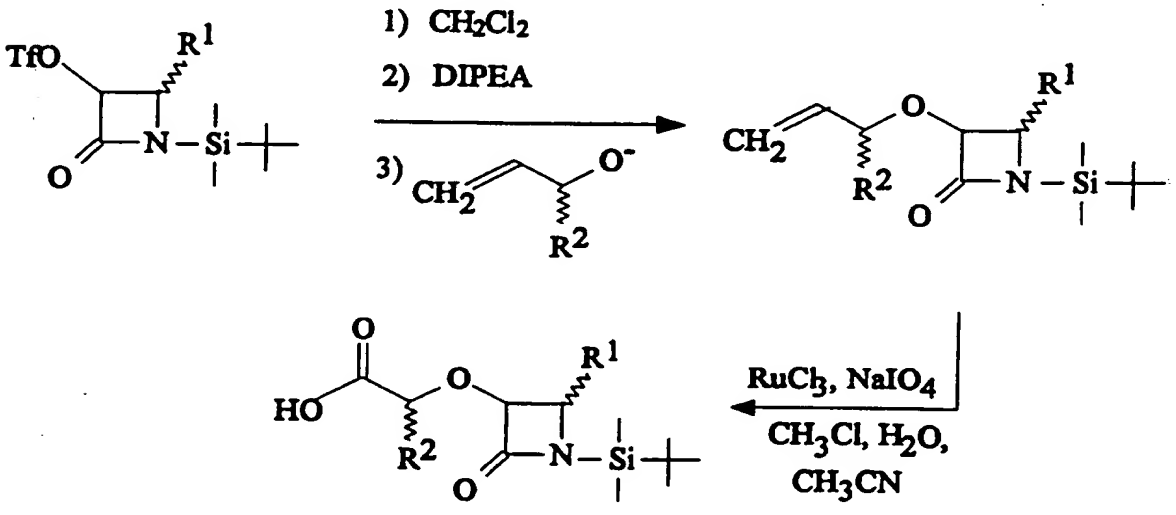
Third component piece 3(a2) may be prepared by the method as generally described in Miller et al. (*J. Amer. Chem. Soc.* 102:7026, 1980). The synthesis of 3(a2) is presented schematically below.



Briefly, the cyclization of a β-hydroxy amide derivative provides an 4-amino-2-azetidinone. Condensation of the azetidinone amino group with an α-keto benzyl ester derivative, and concommitant reduction with sodium cyanoborohydride, yields an ester of 3(a2). Hydrogenolysis of the benzyl protecting group produces third component piece 3(a2).

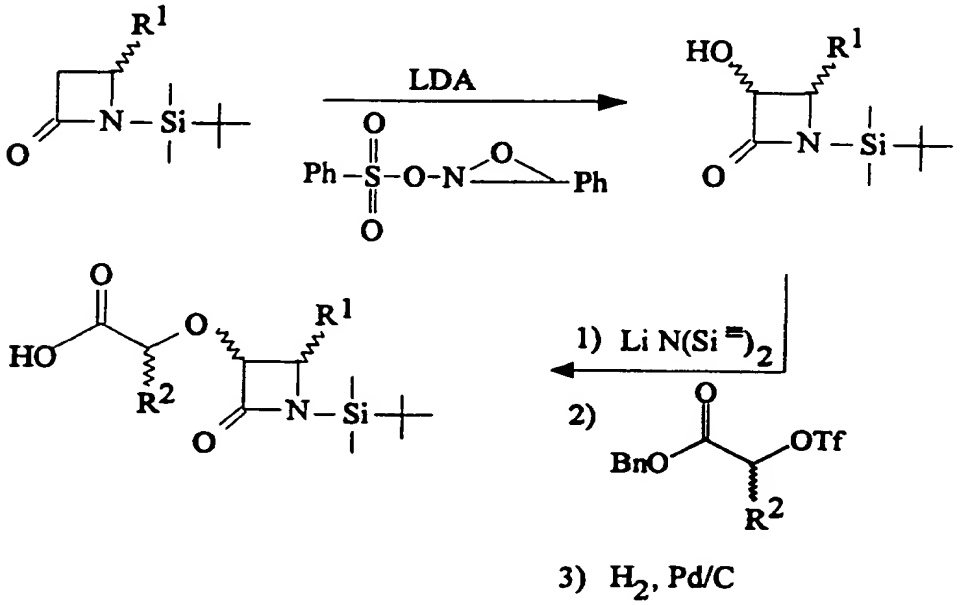
(3) Synthesis of Third Component Piece 3(a3)

Third component piece 3(a3) may be prepared by the method as represented schematically below:



Briefly, displacement of the trifluoromethane sulfonate group from a trifluoromethanesulfonyl-2-TBDMS-2-azetidinone derivative (from hydroxy azetidinc by the oxyanion of an allyl alcohol provides a terminal alkene of 3(a3). Oxida cleavage of the terminal alkene with ruthenium tetroxide provides carboxylic acid 3(a

Alternatively, and in a preferred embodiment, the third component piece 3(a3 prepared by the following procedure:



In the above reaction, hydroxylation of the β-lactam and displacement of corresponding triflate of the hydroxy ester, and subsequent hydrogenolysis, provides desired third component piece.

(4) Synthesis of Third Component Piece 3(a4)

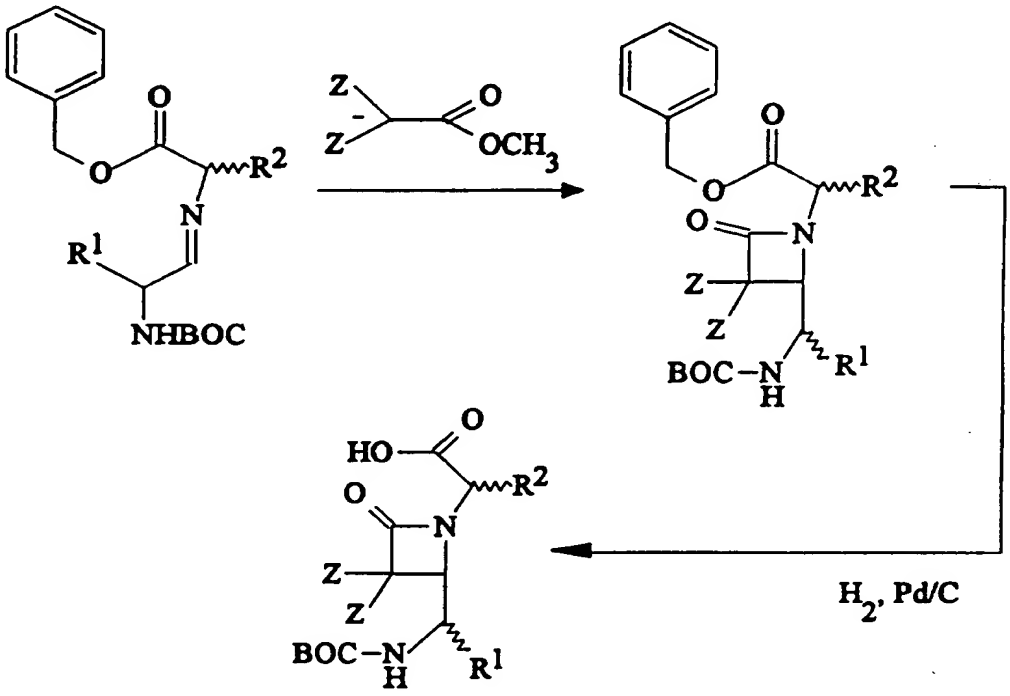
Third modular component piece 3(a4) is made in the same manner as 3(a except $POC(O)CH(R^2)S^-$ is added in place of the allyl alcohol, and the resulting este hydrolysed to yield 3(a4).

(5) Synthesis of Third Component Piece 3b

Third modular component piece 3b is made in the same manner as 3(a3), exc an ester of proline is added in place of the allyl alcohol, and the resulting ester hydrolysed to yield 3(b).

(6) Synthesis of Third Component Piece 3c

Third component piece 3c may be prepared by the method as generally described in Hart and Hu (*Chem. Rev.* 89:1447, 1990). The synthesis of 3c is presented schematically below.



Briefly, the imine product of the condensation of the α-amino group of a amino acid benzyl ester with the carbonyl group of an N-BOC protected aldehyde derived from an α-amino acid was treated with the enolate of methyl isobutyrate to yield a 4,4-dimethyl-2-azetidinone derivative. Hydrogenolysis of the benzyl protecting group provides third component piece 3c where P is a BOC protecting group and Z is methyl.

Alternatively, treatment of the imine above with the enolate of methyl acetate followed by hydrogenolysis provides third component piece 3c where Z is hydrogen.

Example 2

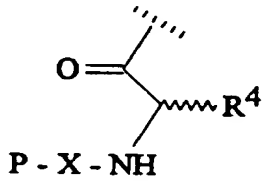
The Coupling of First, Second and/or Third Component Pieces

The coupling of the component pieces to produce the reverse-turn mimetics of the present invention generally involve the formation of amide bonds. The amide bonds which link the pieces may be formed by standard synthetic peptide techniques and may be performed by either liquid or solid phase synthesis.

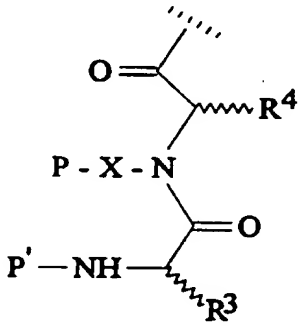
Typically, in the solid phase synthesis of a peptide containing a conformationa constrained reverse-turn mimetic, the first component piece is incorporated into peptide sequence at a specific point in the synthesis. Once the first component piece incorporated, the synthesis of the remainder of the mimetic turn follows. For examp for a conformationally constrained beta-turn mimetic, the turn is synthesized subsequent coupling of a second and a third component piece, followed by cyclization the first component piece to the third component piece. The remainder of the pepti may then be synthesized by further elongation of the peptide chain via continued ste wise coupling of the remaining amino acids.

A. Representative Coupling of First and Second Component Pieces

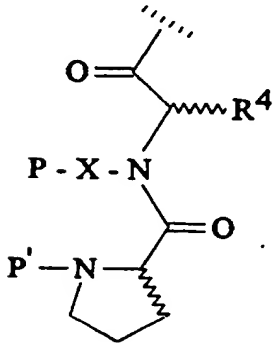
The coupling of the first and second component pieces provides a combined fir second intermediate. Three different combined first-second intermediate species may formed by coupling the first component piece 1 with second component pieces 2a, 2b 2c. The coupling products, identified as 1-2a, 1-2b, and 1-2c, are shown below.



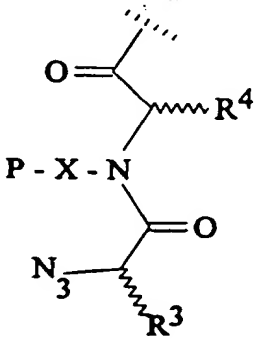
1



1-2a



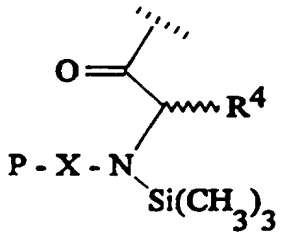
1-2b



1-2c

In the above representation, P is BOC and P' is FMOC.

The coupling of the first and second component pieces may be accomplished by a silicon mediated acid fluoride coupling. In this coupling procedure, solid phase immobilized 1 is converted to the corresponding N-trimethylsilyl derivative (1a below) by treatment with 5 equivalents of bis(trimethylsilyl)acetamide.



1a

Reaction of either of the acid fluorides of 2a-2c with N-silyl derivative 1a yields combined first-second intermediate 1-2a, 1-2b, or 1-2c, respectively. The acid fluorides of 2a-2c may be readily prepared from the corresponding carboxylic acids by cyanuric fluoride treatment according to general method as described by Carpino and Han (*J. Amer. Chem. Soc.* 112:9651-52, 1990).

(1) Peptide Coupling with N-FMOC Protected Amino Acids

The general silicon mediated acid fluoride peptide coupling procedure described above may be utilized to couple N-protected amino acid fluorides (such as 2a or 2b) with N-silyl peptides (such as 1a). For peptide synthesis in the liquid phase, N-protected amino acid fluorides are coupled to carboxy protected N-silyl amino acids. The silicon mediated acid fluoride coupling method represents an advancement over traditional amide forming reactions which employ coupling agents such as carbodiimides or mixed anhydride reagents. These reagents activate the carboxyl group of an amino acid for coupling with the amino group of another amino acid. Alternatively, and in a preferred embodiment, coupling between the amino terminus and carbonyl group can be effected with HATU.

Despite the improvements and advantages that the silicon mediated acid fluoride coupling offers, in some instances, peptide bond formation remains difficult due to the steric interaction between the amino acid side chain of the N-silyl peptide and the traditional bulky N-FMOC (fluorenylmethoxycarbonyl) protecting group of the incoming carboxy activated amino acid. In fact, reaction rates for the coupling of amino acids

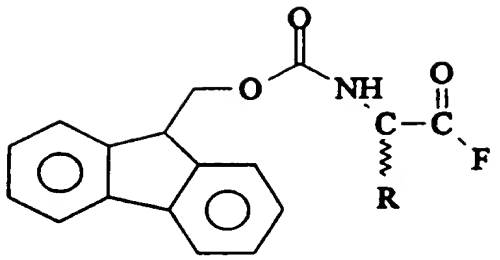
with relatively bulky side chains (such as histidine, phenylalanine, trytophan, tyros arginine, leucine, isoleucine, glutamine, asparagine, aspartic acid, glutamic acid threonine) are significantly less compared with those amino acids having relatively sterically demanding side chains such as glycine, alanine, and serine in coupling reacti which utilize N-FMOC protected amino acids.

Peptide bond formation with secondary amines such as 1a is also more diffi for steric reasons. The additional amino substitutent significantly increases the st hinderance of the amine group and diminishes its nucleophilicity (ability to form pep bond with a carboxy derivative). The diminution of reactivity is manifested in sl reaction rates. This difficulty in peptide coupling is especially true in reactions with FMOC protected amino acids.

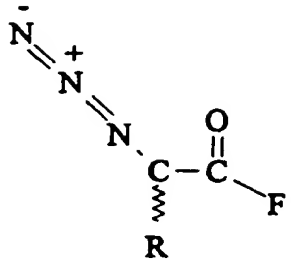
(2) Peptide Coupling with Azido Acid Fluorides

The steric problems associated with peptide coupling of bulky N-FM protected amino acid fluorides can be eliminated by the use of the corresponding az acid fluorides. In contrast to the FMOC group, the azido group is a small (3 nitro atoms), linear substituent. The α-amino group of an amino acid in the former a fluorides are simply protected by a group (FMOC) which prevents interference in peptide coupling reaction. Once coupled, the protecting group is removed subsequent couplings performed. In contrast, in azido acid fluorides, the α-amino gr of an amino acid has been transformed to an azido group (N_3). After coupling with azido acid fluoride, the azido group may be directly activated for subsequent couplin The overall result for either acid fluoride reagent is peptide bond formation. Howe the azido acid fluoride reagent does not suffer from the problems of steric inhibition peptide bond formation associated with N-FMOC protected amino acids.

Accordingly, azido acid fluorides are the reagents of choice for steric demanding peptide bond formation. These couplings include reactions of amino ac with sterically bulky side chain moieties and amide bond formation with second amines. The structures of these acid fluorides is shown below (R represents an am acid side chain moiety or derivative thereof).



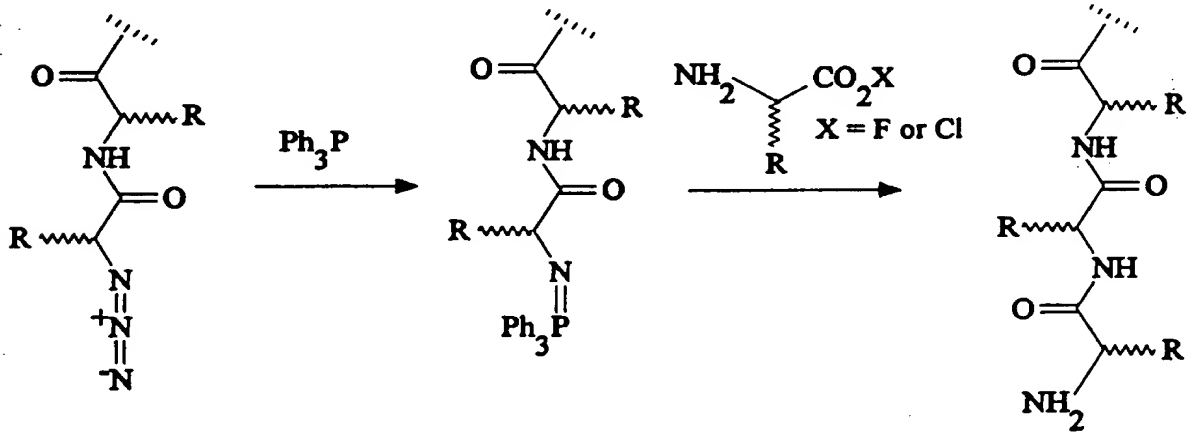
N-FMOC Protected Acid Fluoride



Azido Acid Fluoride

The azido acid fluorides may be prepared from their corresponding azido acids, and the azido acids may be readily synthesized from the corresponding amino acids, by standard methods as disclosed above.

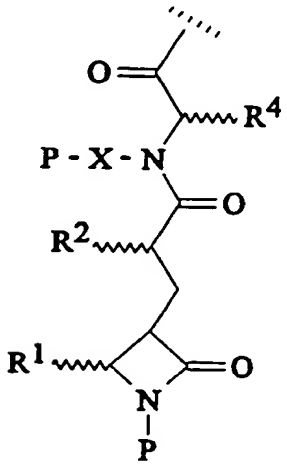
As mentioned above, unlike N-protected amino acid derivatives which must be deprotected prior to subsequent coupling, azido acids may be directly activated for peptide coupling with a subsequent amino acid. Treatment of the azido peptide with triphenyl phosphine generates a phosphine imine which, upon reaction with an amino acid or a second or third component piece of the present invention yields a peptide bond and the corresponding chain lengthened peptide. The process is generally illustrated below where R is an amino acid side chain moiety:



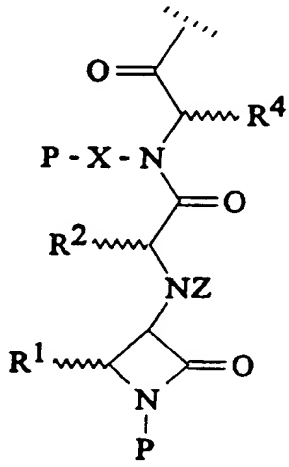
The azido acid fluoride peptide coupling method described above has utility in peptide coupling in general and, more specifically, in the practice of the present invention. This method is further illustrated for coupling of the modular component pieces of the present invention in sections C, D, and E below.

B. Representative Coupling of First and Third Component Pieces

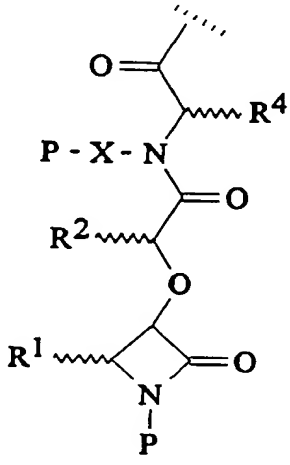
The coupling of the first modular component and third modular compon pieces provides a combined first-third intermediate. Six different combined first-t intermediate species may be formed as result of the coupling of first component piec with either third component piece 3(a1), 3(a2), 3(a3), 3(a4), 3b, or 3c. The coupl products, identified as 1-3(a1), 1-3(a2), 1-3(a3), 1-3(a4), 1-3(b), and 1-3(c), are sho below:



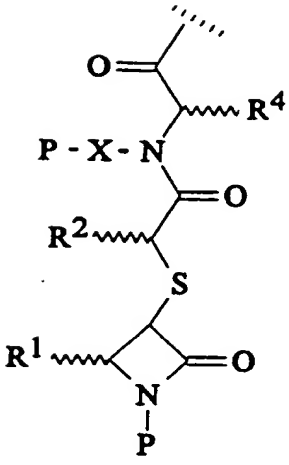
1-3(a1)



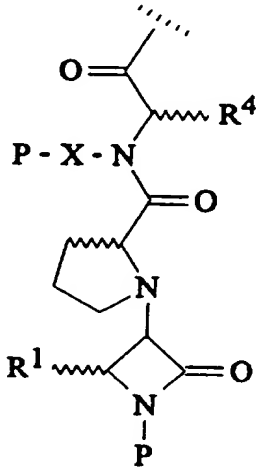
1-3(a2)



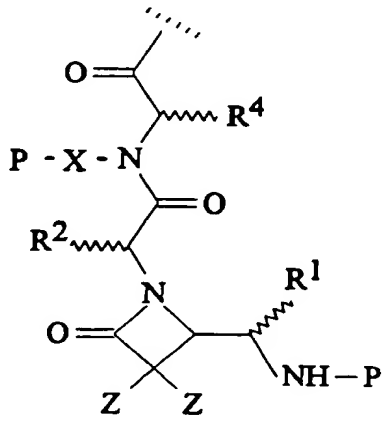
1-3(a3)



1-3(a4)



1-3(b)



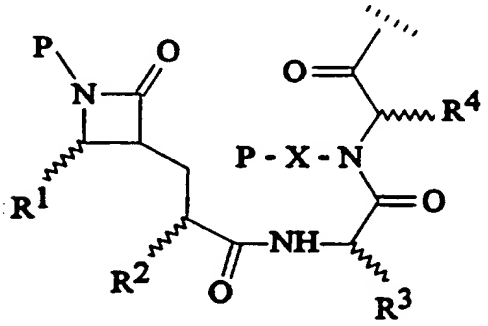
1-3(c)

In the above representation, P is a protective group suitable for use in peptide synthesis, X is a linker as described in Example 1, and R^1, R^2, R^3 and R^4 represent an amino acid side chain moiety.

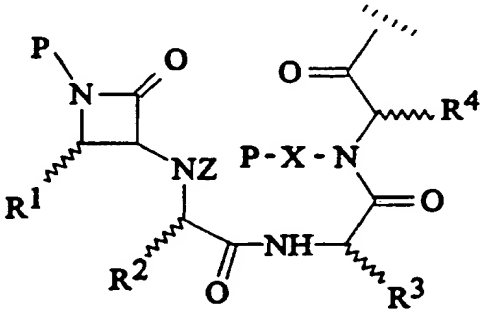
The coupling of the first and third component pieces is accomplished by the silicon mediated acid fluoride method as described in section A above via N-silyl derivative 1a. The combined first-third intermediates are precursors for conformationally constrained gamma-turn mimetic synthesis.

C. Representative Coupling of a Combined First-Second Component Intermediate with a Third Component Piece

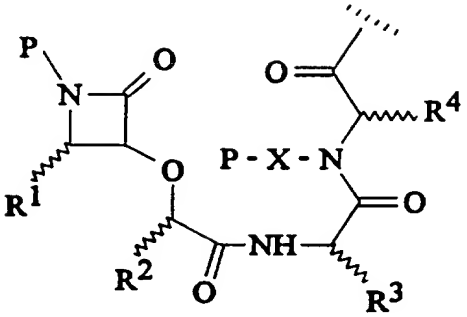
The coupling of a combined first-second intermediate with a third modular component piece provides a combined first-second-third intermediate. Eighteen different combined first-second-third intermediate species may be formed as result of coupling each of the three first-second intermediates 1-2(a), 1-2(b), or 1-2(c) with each of the six third component pieces 3(a)-3(c). The products of coupling 1-2(a) with 3(a)-3(c), a representative set of first-second-third intermediate coupling products, identified as 1-2(a)-3(a1), 1-2(a)-3(a2), 1-2(a)-3(a3), 1-2(a)-3(a4), 1-2(a)-3(b), and 1-2(a)-3(c), are shown below:



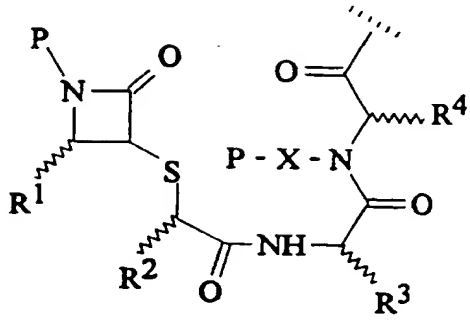
1-2(a)-3(a1)



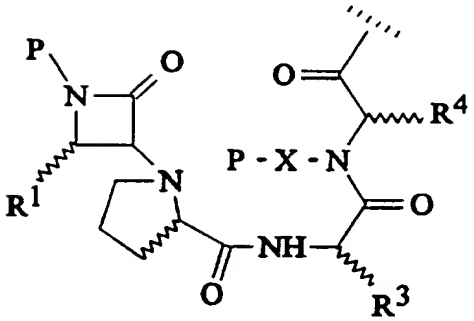
1-2(a)-3(a2)



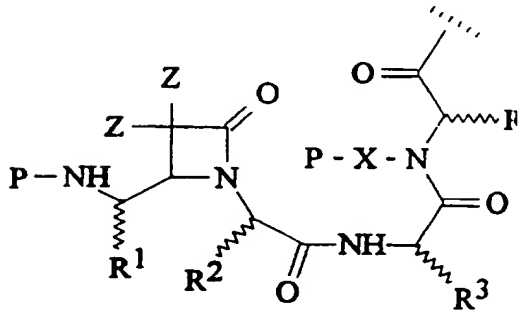
1-2(a)-3(a3)



1-2(a)-3(a4)



1-2(a)-3(b)



1-2(a)-3(c)

In the above structures, P is a protective group suitable for use in pep synthesis, X is a linker as described in Example 1, Z is either hydrogen or methyl, an represents an amino acid side chain moiety. The remaining ten first-second-t intermediates, formed from coupling of 1-2(b) and 1-2(c) with 3(a)-3(c), would h structures analogous to those represented above.

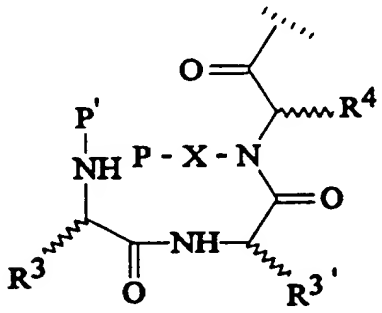
For couplings involving 1-2(b) derivatives, the coupling of a combined fi second intermediate with a third component piece may be accomplished by traditic peptide coupling techniques such as those performed on automated peptide synthesiz Briefly, the steps include N-deprotection of the combined first-second intermedi addition and carboxyl group activation of the third component piece which result peptide bond formation and coupling.

Alternatively, for couplings which involve 1-2(c) derivatives, the couplings readily achieved via triphenyl phosphine activation as described above in section A(2).

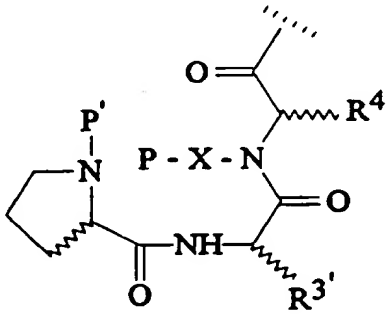
The combined first-second-third intermediates are precursors required conformationally constrained beta-turn mimetic synthesis.

D. Representative Coupling of a Combined First-Second Component Intermediate with a Second Component Piece

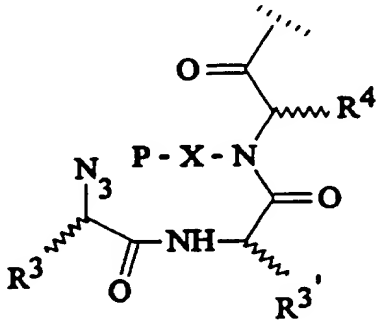
The coupling of a combined first-second intermediate with a second modular component piece provides a combined first-second-second intermediate. Nine different combined first-second-second intermediate species may be formed as result of the coupling each of the three first-second intermediates 1-2(a), 1-2(b), or 1-2(c) with each of the three second component pieces 2(a)-2(c). The products of coupling 1-2(a) with 2(a)-2(c), a representative set of first-second-second intermediate coupling products, identified as 1-2(a)-2(a), 1-2(a)-2(b), and 1-2(a)-2(c), are shown below:



1-2(a)-2(a)



1-2(a)-2(b)



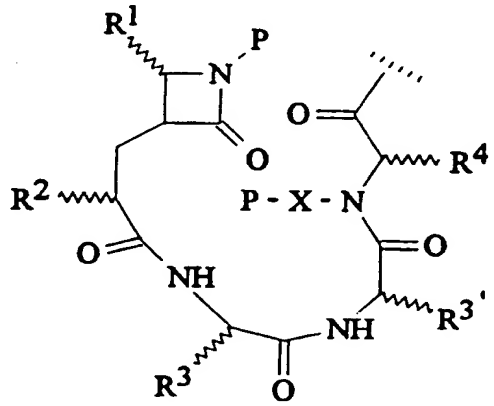
1-2(a)-2(c)

In the above representation, P is a protective group suitable for use in peptide synthesis, X is a linker as described in Example 1, and R represents an amino acid side chain moiety. The remaining first-second-second-third intermediates, formed from coupling of 1-2(b) and 1-2(c) with 2(a)-2(c), would have structures analogous to those represented above.

The coupling of a combined first-second intermediate with a second component piece may be accomplished by the peptide coupling techniques described in C above.

E. Representative Coupling of a Combined First-Second-Second Compon Intermediate with a Third Component Piece

The coupling of a combined first-second-second intermediate with a th modular component piece provides a combined first-second-second-third intermedi Forty-five different combined first-second-second-third intermediate species may formed as result of the coupling each of the nine first-second-second intermediates 2(a)-2(a), 1-2(a)-2(b), 1-2(a)-2(c), 1-2(b)-2(a), 1-2(b)-2(b), 1-2(b)-2(c), 1-2(c)-2(a), 2(c)-2(b), and 1-2(c)-2(c) with each of the five third component pieces 3(a)-3(c). T products of coupling 1-2(a)-2(a) with 3(a1), a representative species of first-seco second-third intermediate coupling products, identified as 1-2(a)-2(a)-3(a1), is sho below:



1-2(a)-2(a)-3(a1)

In the above representation, P is a protective group suitable for use in peptide synthesi X is a linker as described in Example 1, and R represents an amino acid side cha moiety. The remaining first-second-second-third intermediates, would have structur analogous to those represented above.

The coupling of a combined first-second-second intermediate with a thi component piece may be accomplished by the peptide coupling techniques described i C above.

The combined first-second-second-third intermediates are precursors required fc conformationally constrained beta-bulge mimetic synthesis.

Example 3

Cyclization of Combined Component Pieces to Form Conformationally Constrained Reverse-Turn Mimetics

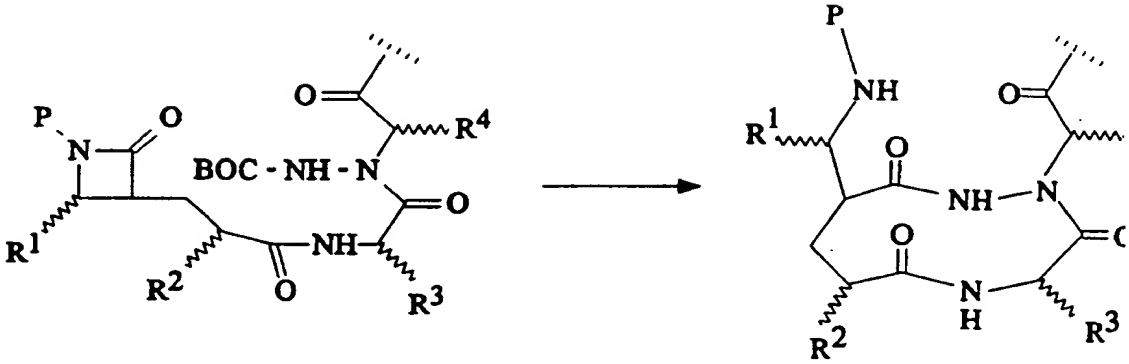
The cyclization of the first and third modular component pieces through covalent coupling of linking group X of the first component piece with the carbonyl carbon of the azetidinone group of the third component piece yields the conformationally constrained reverse-turn mimetics of the present invention. The presence of component pieces between the first and third component pieces determines the nature of the conformationally constrained reverse-turn mimetic (i.e., beta-turn, gamma-turn or beta-bulge mimetic).

Cyclization of the first component piece to the third component piece involves removal of protecting group P from linking group X of the first component piece. As described in Example 1A, P is an amino protective group, typically a BOC group. Therefore, treatment of the combined intermediate with TFA in CH_2Cl_2 and subsequent neutralization with DIPEA removes the protecting group and provides linking group X, which bears a nucleophilic nitrogen. Depending upon the nature of X, the nucleophilic nitrogen is either a hydrazine, Example 1A(1), or an amine, Example 1A(2)(a-d).

The nucleophilic hydrazine or amine of the first component piece results in facile cyclization to the hydrazide or amide, respectively. The ease of cyclization is due to the proximity and the electrophilic nature of the azetidinone carbonyl of the third component piece.

A. Representative Conformationally Constrained Beta-Turn: Cyclization of a Combined First-Second-Third Modular Component

N-deprotection and cyclization of the first-second-third combined intermediate provides the beta-turn mimetic. The combined intermediate may be any one of those described above in Example 2C. For example, aqueous acid treatment of 1-2(a)-3(a1), for a species where X = -NH-, results in cyclization to the conformationally constrained beta-turn mimetic shown below:

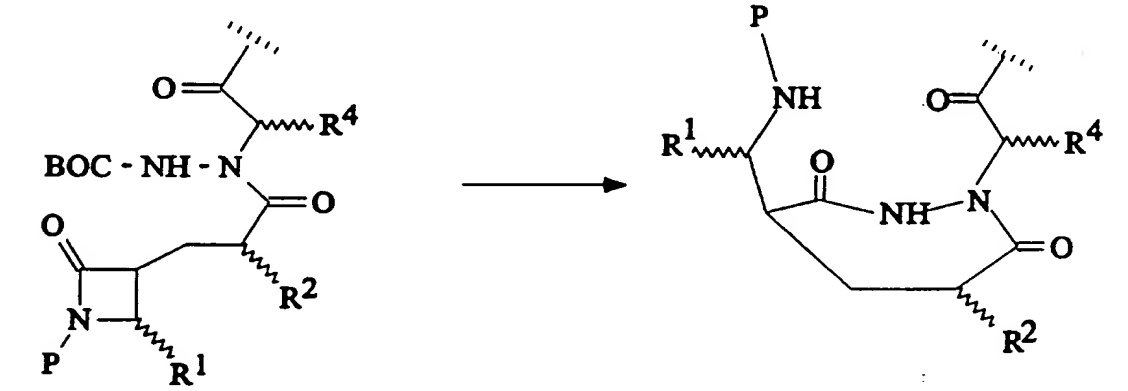


1-2(a)-3(a1)

The cyclization depicted above is representative for all beta-turn mimetic cyclization the present invention.

B. Representative Conformationally Constrained Gamma-Turn Mimetic: Cyclization of a Combined First-Third Modular Component

N-deprotection and cyclization of a first-third combined intermediate provi the gamma-turn mimetic. The combined intermediate may be any one of those descri above in Example 2B. For example, aqueous acid treatment of 1-3(a1), for a spe where X = -NH-, results in cyclization to the conformationally constrained gamma-t mimetic shown below:

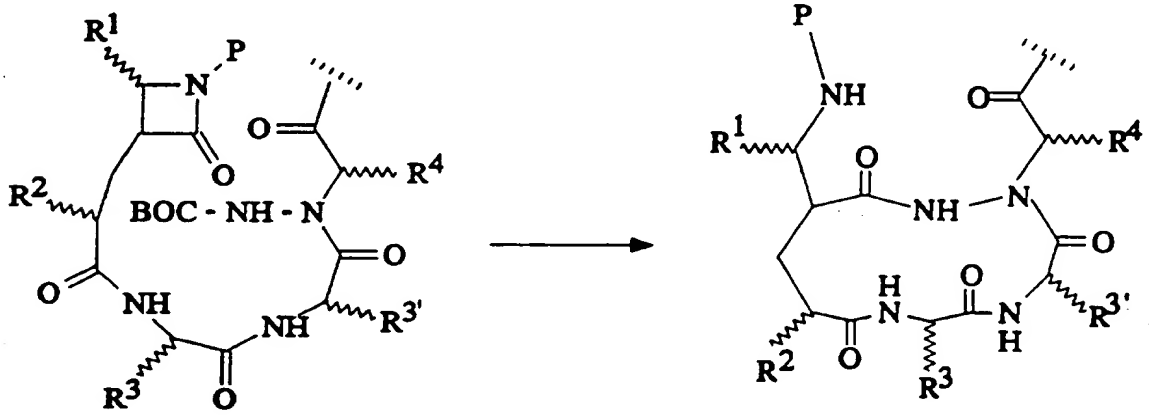


1-3(a1)

The cyclization depicted above is representative for all gamma-turn mimetic cyclizati of the present invention.

C. Representative Conformationally Constrained Beta-Bulge Mimetic: Cyclization of a Combined First-Second-Second-Third Modular Component

N-deprotection and cyclization of a first-second-second-third combined intermediate provides the beta-bulge mimetic. The combined intermediate may be any one of those described above in Example 2E. For example, TFA/CH_2Cl_2 treatment and neutralization of 1-2(a)-2(a)-3(a1), for a species where X = -NH-, results in cyclization to the conformationally constrained beta-bulge mimetic shown below.



1-2(a)-2(a)-3(a1)

The cyclization depicted above is representative for all beta-bulge mimetic cyclizations of the present invention.

Example 4

Synthesis of a Representative Library Containing Conformationally Constrained Reverse-Turns which Mimetic Beta-Endorphin and Identification of a Bioactive Mimetic Therefrom

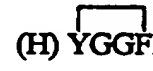
In preferred embodiment of this invention, the sequence of a linear peptide having known biological activity is obtained. Such a sequences may be of a naturally occurring peptide, a sequence that was identified from a linear peptide library (e.g., synthetic or phage display), or a combination of epitopes that are part of a bioactive region of a protein. In this example, the linear peptide is beta-endorphin having the following sequence:

H-YGGFM TSEKS QTPLV TLFRN AIIKN AYKKG E-OH

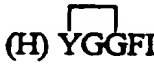
The N-terminal sequence of the above peptide (*i.e.*, YGGFM(L)) is recognized by anti-beta-endorphin antibody, 3E7 (Boehringer Mannheim) (Lam et al., *Nature* 354: 84, 1991) (incorporated herein by reference).

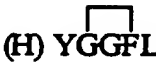
A mimetic library is constructed around the N-terminal, pentapeptide sequence determine its bound (*i.e.*, bioactive) conformation as follows:

Beta-Turn Mimetics:

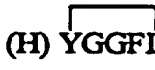


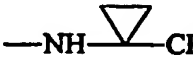
Gamma-Turn Mimetics :





Beta-Bulge Mimetics:



Different X moieties are utilized (*e.g.*, -NH-, -NHCH(CH_3)CH -NHC(CH_3)$_2$CH=CHCH_2-,  and -NHC(CH_3)$_2$CH_2CH_2CH_2 resulting in beta-turn mimetics having 10-, 12- and 14-membered rings, gamma-tu mimetics having 7-, 9- and 11-membered rings, and beta-bulge mimetics having 13-, and 17-membered rings (*e.g.*, (2x5) + (3x5) + (1x5) = 30 members).

Synthesis of the above mimetics is generally achieved by the procedu disclosed in Examples 1-3 above, and illustrated in Figures 1 and 2. In Figures 1A a 1B, synthesis of the beta-turn mimetics having 12- and 14-membered rings are disclos while Figures 2A, 2B and 2C illustrate synthesis of the gamma-turn mimetic having a membered ring and a beta-turn mimetic having a 10-membered ring are disclos Synthesis of gamma-turns having 9- and 11-membered rings, and beta-bulges having 1 and 17-membered rings follows the synthesis illustrated in Figures 1A and 1B, except f the omission of the second component piece (gamma-turns) or addition of a furth second component piece (beta-bulges). Synthesis of the beta-bulge containing a 1 membered ring follows the disclosure of Figures 2A, 2B and 2C, except a further seco modular component piece is included.

More specifically, the first step involves coupling commercially available BO Gly-PAM resin to the first two amino acids (i.e., K and L) by DIC, HOBT (i. carbodiimide coupling) to yield FMOC-LKG-PAM. In the case of the beta-tu mimetics having 12- and 14-membered rings (and similarly for the gamma-turn mimeti having 9- and 11- membered rings and the beta-bulge mimetics having 15- and 1 membered rings), the next step is reductive amination on the resin to complete t

synthesis of the linker, X. In the case of the beta-turn mimetic having a 10-membered ring (and similarly for a gamma-turn mimetic having a 7-membered ring and a beta-bulge mimetic having a 13-membered ring), the amino acid hydrazide is presynthesized (i.e., the first component piece). Coupling of the second component piece to the hindered secondary amine is effected using HATU (4x). Removal of the FMOC group and coupling to the beta-lactam (i.e., the third component piece) is the same as outlined above in Example 2, and cyclizing the ring with TFA is accomplished by the methods disclosed in Example 3. A further amino acid may be added using HATU, with additional residues added via carbodiimide coupling. Cleavage of the reverse-turn mimetics from the resin is effected by aminolysis (NH_3 in isopropanol).

The library members are then assayed in a standard ELISA format. Briefly, Costar ELISA strips (2588) are coated with 1μg/ml of beta-endorphin (Novabiochem) of carbonate buffer pH 8.6 at 50μg/well for 1 hour at room temperature, and then washed three times with PBS and 0.5% Tween 20. The wells are filled (about 350 μl) with blocking buffer (PBS, 1% BSA, and 0.05% Tween 20) and incubated at room temperature for 2 hours and washed three times.

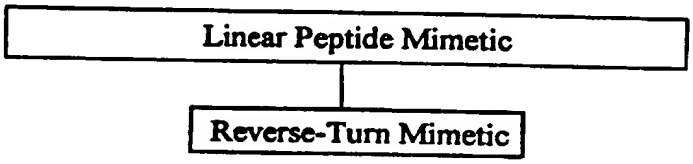
Anti-beta-endorphin antibody (3E7) is diluted to 150 ng/ml in blocking buffer, and the library members are incubated with 3E7 at varying concentration for 30 minutes at room temperature. Then 100 μl of each mixture is applied to the wells and incubated 30 minutes to 1 hour at room temperature. The linear peptide YGGFLKG is tested similarly as a positive control. After 1 hour, the wells are washed three times and then incubated with Goat-anti-Mouse (Sigma) HRP conjugated antibody at 1:1000 dilution in blocking buffer (100 μl/well) and incubated for 30 minutes to 1 hour at room temperature and washed three times. ABTS (Sigma) is dissolved at 1μg/ml in 0.1M citrate buffer (pH 4.0) to which is added 10 μl of 30% H_2O_2 per ml of solution. 100 μl of ABTS solution is added per well, incubated at room temperature for 30 minutes, and the optical density is read at 415 nM.

The most inhibitory reverse-turn mimetic is indicated by the well having the lowest optical density. Once having determined the reverse-turn mimetic template, the above procedure is then repeated to yield a library containing reverse-turn mimetics having the same template, but having differing amino acid side chain moieties to determine the most biologically active. In other words, the side chains of the template are optimized by randomizing the amino acid residues, particular the residues of the ring. Further modification may also be made, such as modifying the amino terminus by, for example, acylation, or by utilizing a mixture of D and L configurations, including an all D configuration.

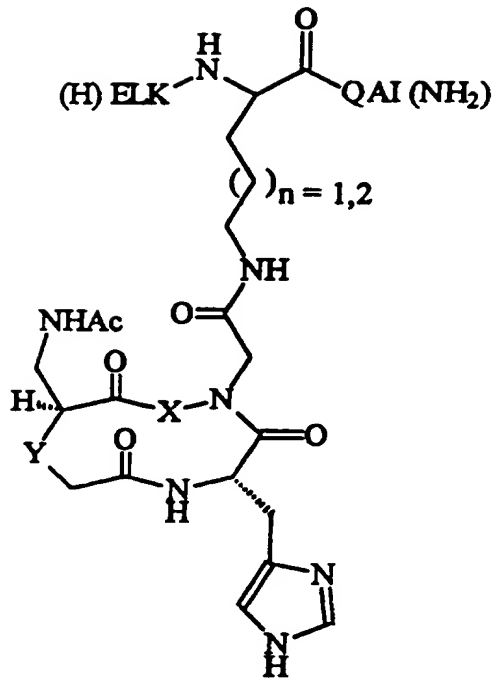
Example 5
Synthesis of a Representative Library Containing Conformationally Constrained Reverse-Turns which Mimetic IL-8 and Identification of a Bioactive Mimetic Therefrom

IL-8 is a member of the chemokine superfamily, and has been implicated inflammation, angiogenesis and neutrophil chemotaxis. Based on structural (Baldwir al., *Proc. Natl. Acad. Sci.* **88**:502-505, 1991) and mutational analysis (Clark-Lewis al., *J. Biol. Chem.* **269**:16075-16081, 1994) the region of IL-8 involved in binding to receptor is believed to encompass both the N-terminal residues 4-10 (ELRCQCI) a the loop containing Cys-34 (GPHC). In other words, the IL-8 binding region is belie to involve a linear peptide sequence closely associated with, in three-dimensional spa a reverse-turn structure.

To mimic this binding region, a reverse-turn of the present invention is joined a linear peptide as represented by the following:



In the above representation, the linear peptide (or mimetic thereof) is covalently join to one end of a reverse turn mimetic of this invention. In the case of IL-8, a libra containing the following structures are synthesized:



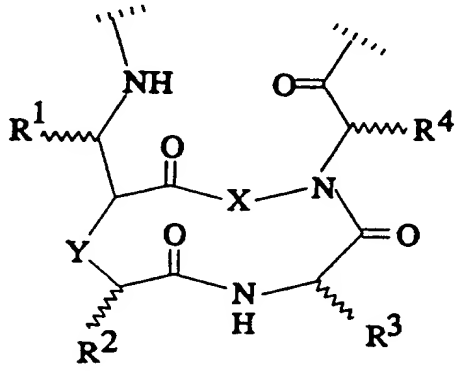
A library of the above mimetics is constructed in the manner disclosed above in Examples 1-4, with the exception of the linear peptide joined to the carbonyl terminus of the reverse-turn. In short, the peptide IAQ is constructed on PAM resin to yield PAM-IAQ-FMOC. A DDE (ε-amino FMOC) lysine residue (Bycroft et al., *J. Amer. Chem. Soc.* 116:7415-7416, 1994) is then coupled thereto. The FMOC group is removed with diethylamine and the synthesis of the reverse-turn proceeds as disclosed by the previous examples. Subsequently, the DDE group is removed with 2% N_2H_4 at room temperature, and the KLE residues are individually added using carbodiimide coupling. Cleavage from the resin is effected as described above.

The resulting library is screened by determination of the inhibition of IL-8 included elastase release from human neutrophils which have been pretreated with cytochalasin B. Such an assay is disclosed in Peveri et al., *J. Exp. Med.* 167:1547-1557, 1988 (incorporated herein by reference). The library may also be screened by other procedures, including the assay disclosed in Graminski and Lerner, *Biotechnology* 12:1008-1011, 1994 (incorporated herein by reference).

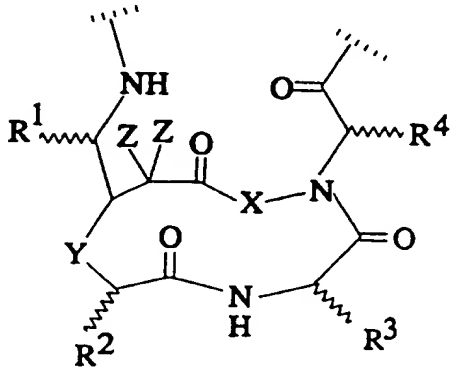
From the foregoing, it will be appreciated that, although specific embodiments of this invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the invention is not limited except by the appended claims.

Claims

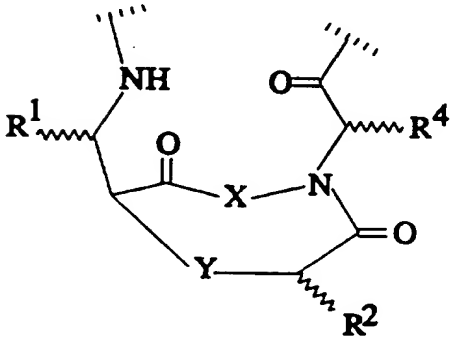
1. A library containing a plurality of conformationally-constrained rev turn mimetics as members, wherein the mimetics are selected from the following structur II, III, IV, V and VI:



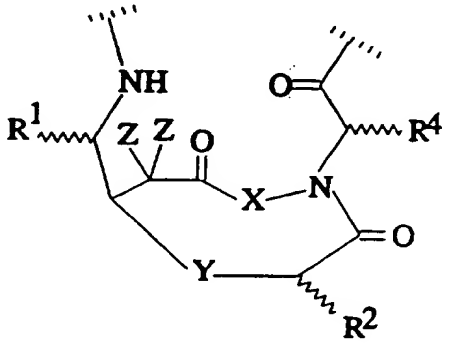
I



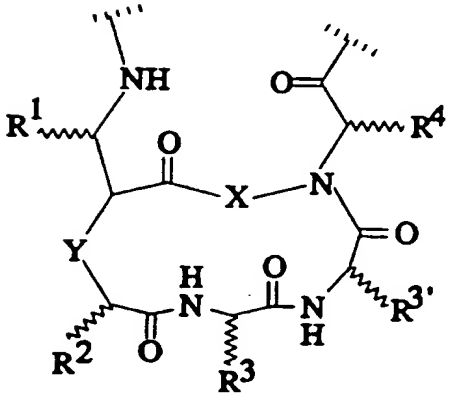
II



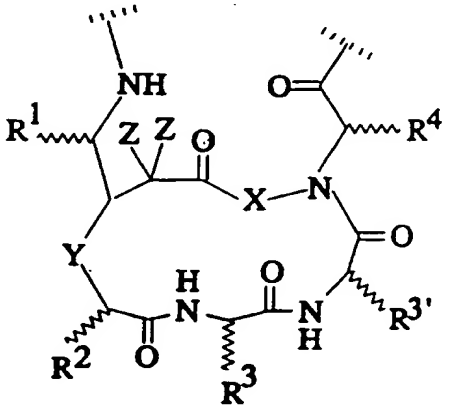
III



IV



V



VI

wherein R^1, R^2, R^3, $R^{3'}$ and R^4 are amino acid side chain moieties, X is selected from the chemical moieties identified in Table 1, Y is selected from $-CH_2-$, -N(Z)-, -O- and -S-, and Z is -H or $-CH_3$.

2. A method for identifying a biologically active agent, comprising screening the library members of claim 1 in a suitable assay and thereby identifying the biologically active library member.

3. A method for identifying a biologically active conformationally constrained reverse-turn mimetic which mimics a biologically active conformation of a linear peptide having a known amino acid sequence, comprising:

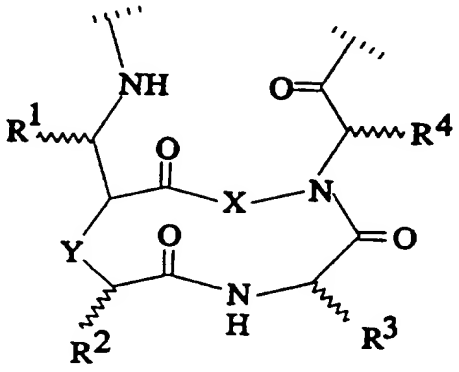
constructing conformationally constrained reverse-turn mimetics based on the known amino acid sequence of the linear peptide to yield a template library, wherein the template library contains a plurality of members selected from the group consisting of conformationally constrained beta-turn, gamma-turn and beta-bulge mimetics having multiple ring sizes;

screening the template library members in a suitable assay to identify a biologically active member of the template library;

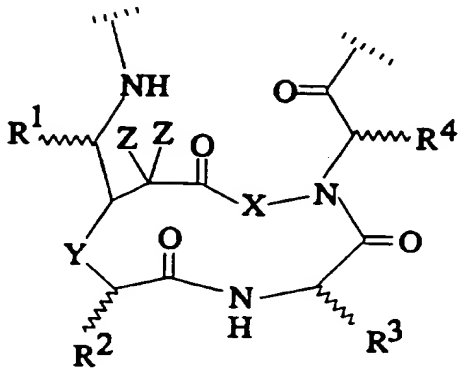
constructing conformationally constrained reverse-turn mimetics based on the biologically active member of the template library to yield an optimized library, wherein the optimized library contains a plurality of members having varying amino acid substitutions based on the known amino acid sequence of the biologically active linear peptide; and

screening the optimized library members in a suitable assay to identify a biologically active member of the optimized library.

4. A conformationally constrained reverse-turn mimetic having following structure I or II:



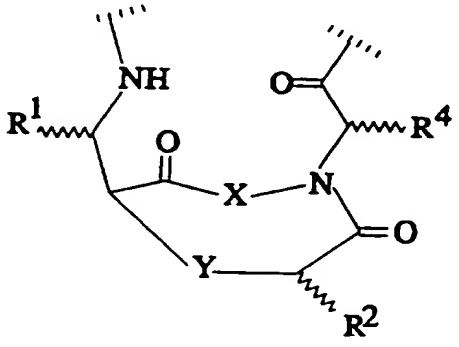
I



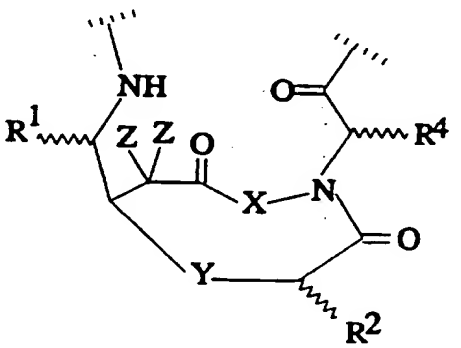
II

wherein R^1, R^2, R^3 and R^4 are amino acid side chain moieties, X is selected from tl chemical moieties identified in Table 1, and Y is selected from -O- and -S-.

5. A conformationally constrained reverse-turn mimetic having tl following structure III or IV:



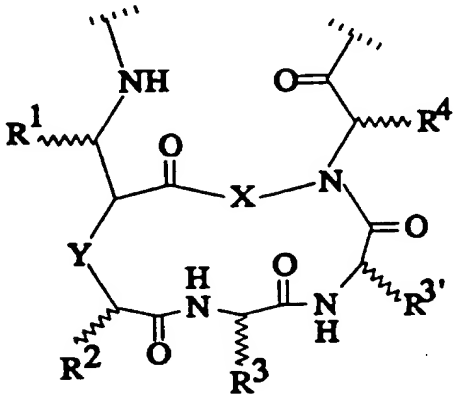
III



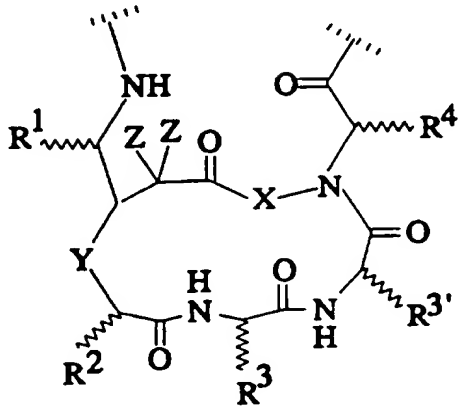
IV

wherein R^1, R^2 and R^4 are amino acid side chain moieties, X is selected from the chemica moieties identified in Table 1, and Y is selected from -O- and -S-.

6. A conformationally constrained reverse-turn mimetic having the following structure V or VI:



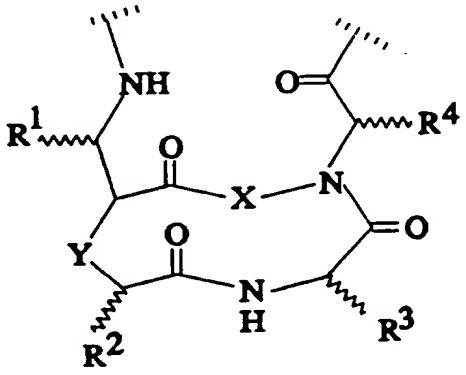
V



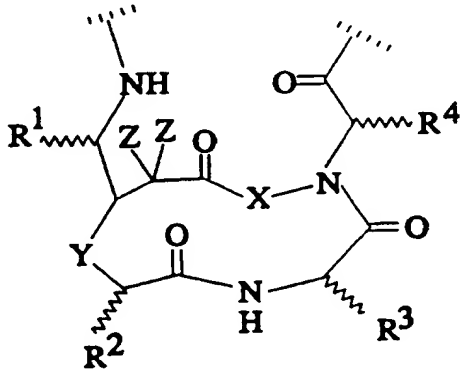
VI

wherein R^1, R^2, R^3, $R^{3'}$ and R^4 are amino acid side chain moieties, X is selected from the chemical moieties identified in Table 1, and Y is selected from -O- and -S-.

7. A conformationally constrained reverse-turn mimetic having the following structure I or II:

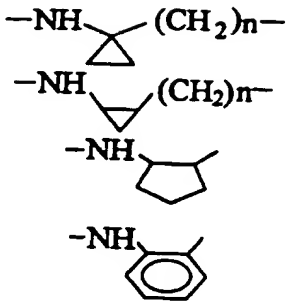


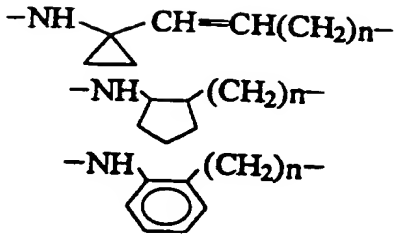
I



II

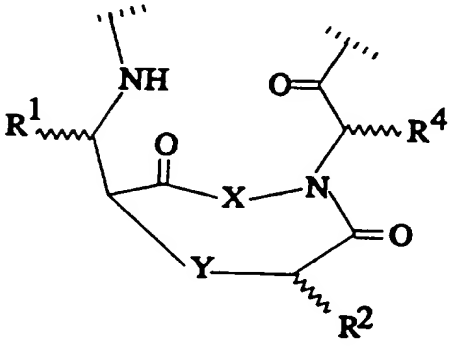
wherein R^1, R^2, R^3 and R^4 are amino acid side chain moieties, X is selected from



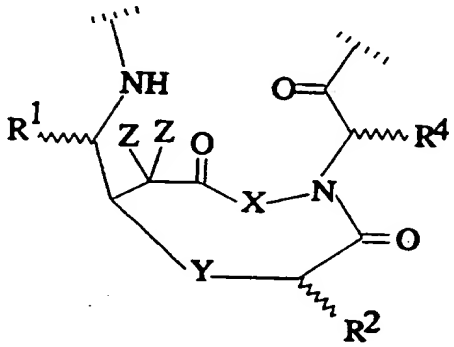


wherein n=1-4, and Y is selected from -CH_2- and -NH-.

8. A conformationally constrained reverse-turn mimetic having t following structure III or IV:

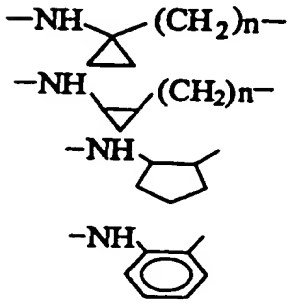


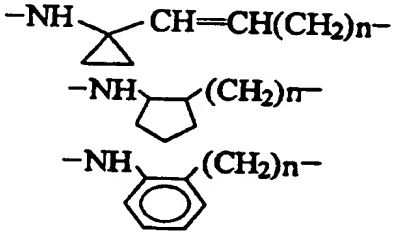
III



IV

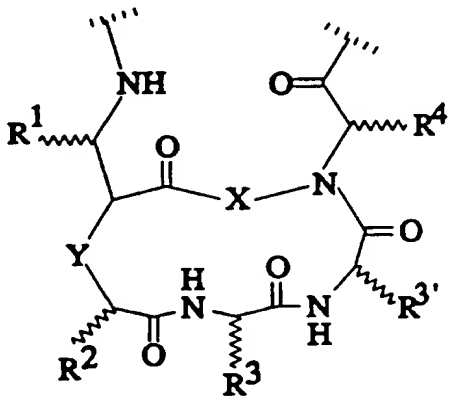
wherein R^1, R^2, and R^4 are amino acid side chain moieties, X is selected from



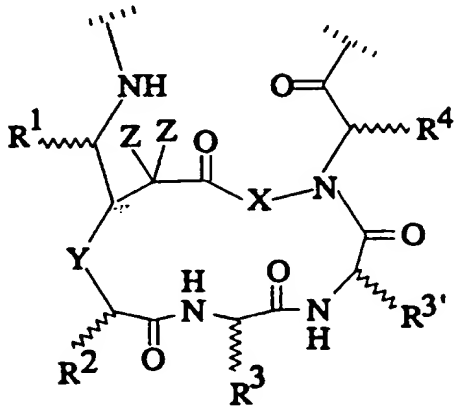


wherein n=1-4, and Y is selected from -CH_2- and -NH-.

9. A conformationally constrained reverse-turn mimetic having the following structure V or VI:

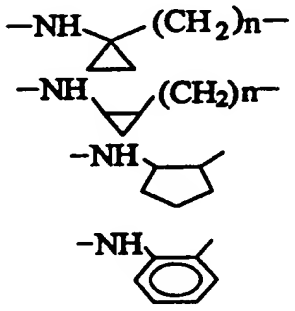


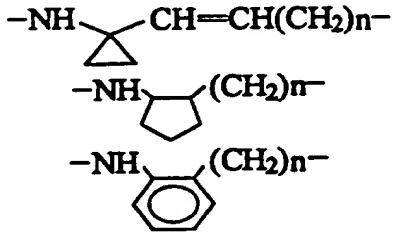
V



VI

wherein R^1, R^2, R^3, $R^{3'}$ and R^4 are amino acid side chain moieties, X is selected from





wherein n=1-4, and Y is selected from -CH_2- and -NH-.

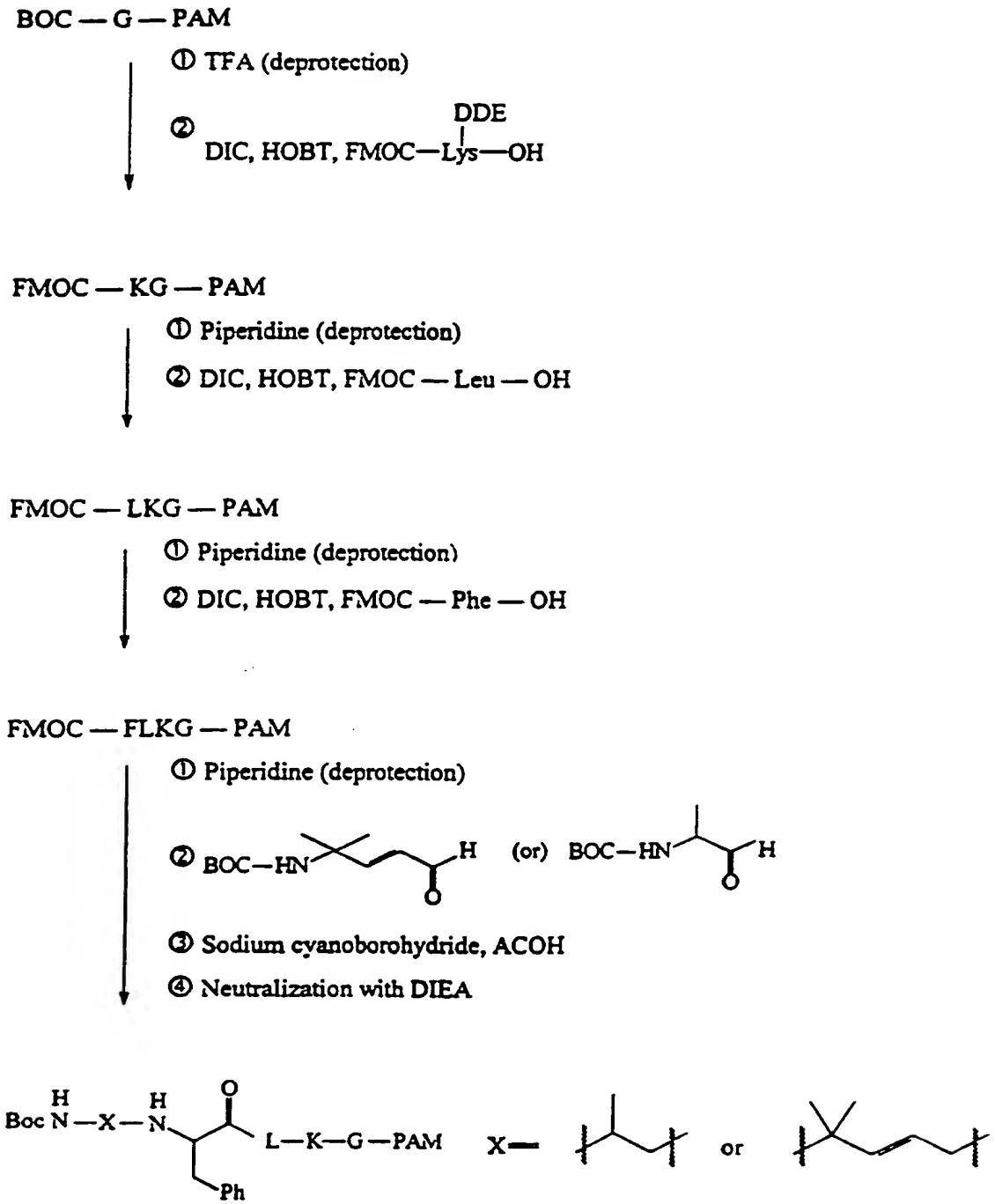

BOC — G — PAM
① TFA (deprotection)
② DIC, HOBT, FMOC—Lys(DDE)—OH
FMOC — KG — PAM
① Piperidine (deprotection)
② DIC, HOBT, FMOC — Leu — OH
FMOC — LKG — PAM
① Piperidine (deprotection)
② DIC, HOBT, FMOC — Phe — OH
FMOC — FLKG — PAM
① Piperidine (deprotection)
② BOC—HN H (or) BOC—HN H
③ Sodium cyanoborohydride, ACOH
④ Neutralization with DIEA
Boc N—X—N L—K—G—PAM
Ph
X—
or

FIG. 1A

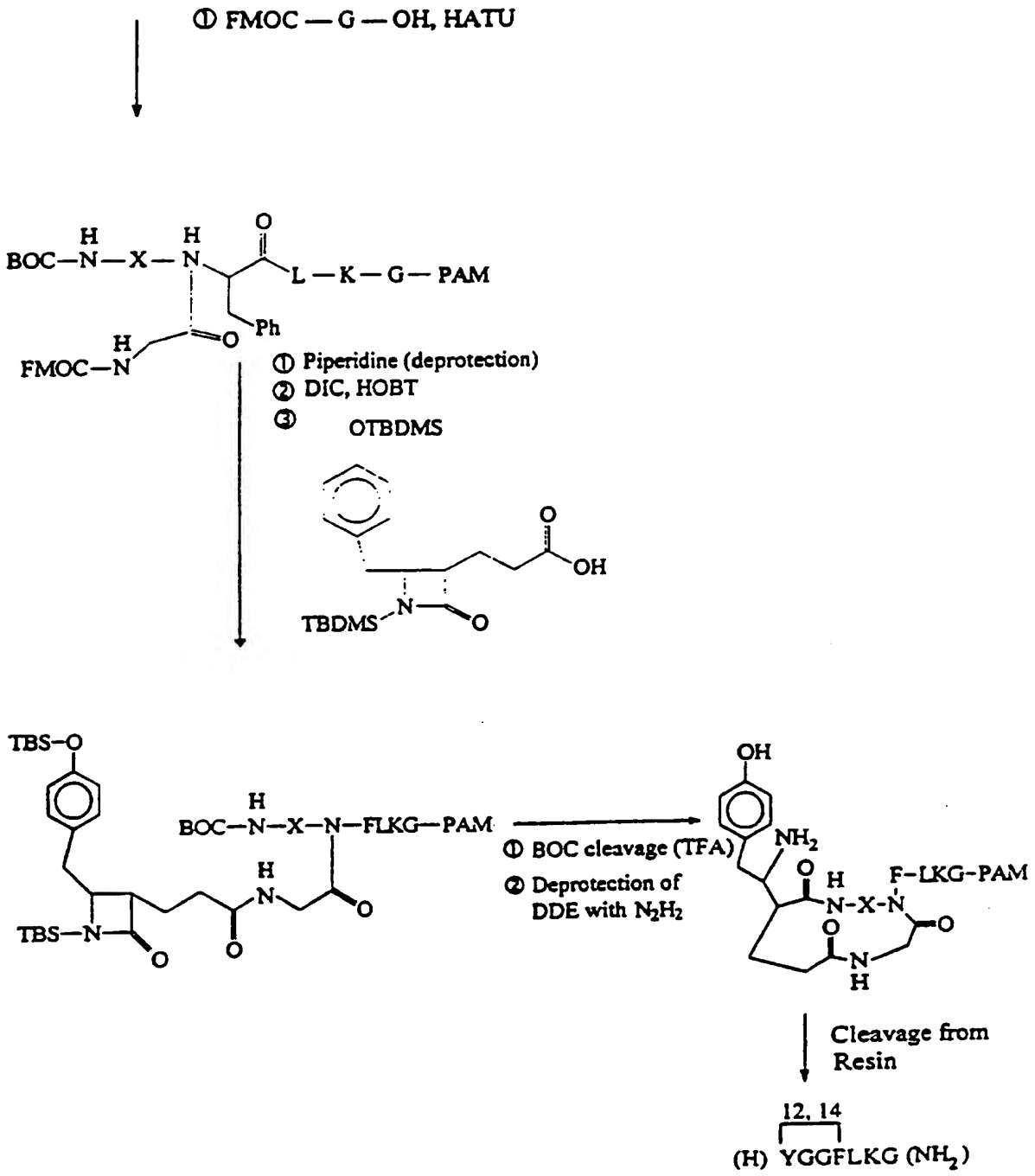

① FMOC — G — OH, HATU
BOC—N—X—N
L—K—G—PAM
Ph
FMOC—N
① Piperidine (deprotection)
② DIC, HOBT
③ OTBDMS
TBDMS
OH
TBS—O
BOC—N—X—N—FLKG—PAM
TBS—N
① BOC cleavage (TFA)
② Deprotection of DDE with N_2H_2
NH2
F-LKG-PAM
Cleavage from Resin
12, 14
(H) YGGFLKG (NH_2)

FIG. 1B

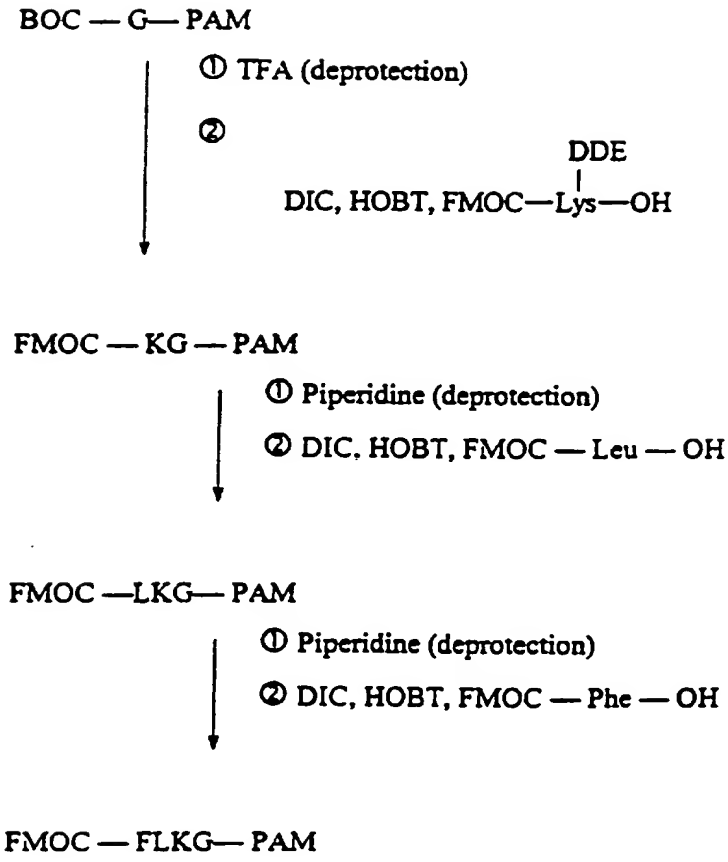

BOC — G— PAM
① TFA (deprotection)
②
DDE
DIC, HOBT, FMOC—Lys—OH
FMOC — KG — PAM
① Piperidine (deprotection)
② DIC, HOBT, FMOC — Leu — OH
FMOC —LKG— PAM
① Piperidine (deprotection)
② DIC, HOBT, FMOC — Phe — OH
FMOC — FLKG— PAM

FIG. 2A

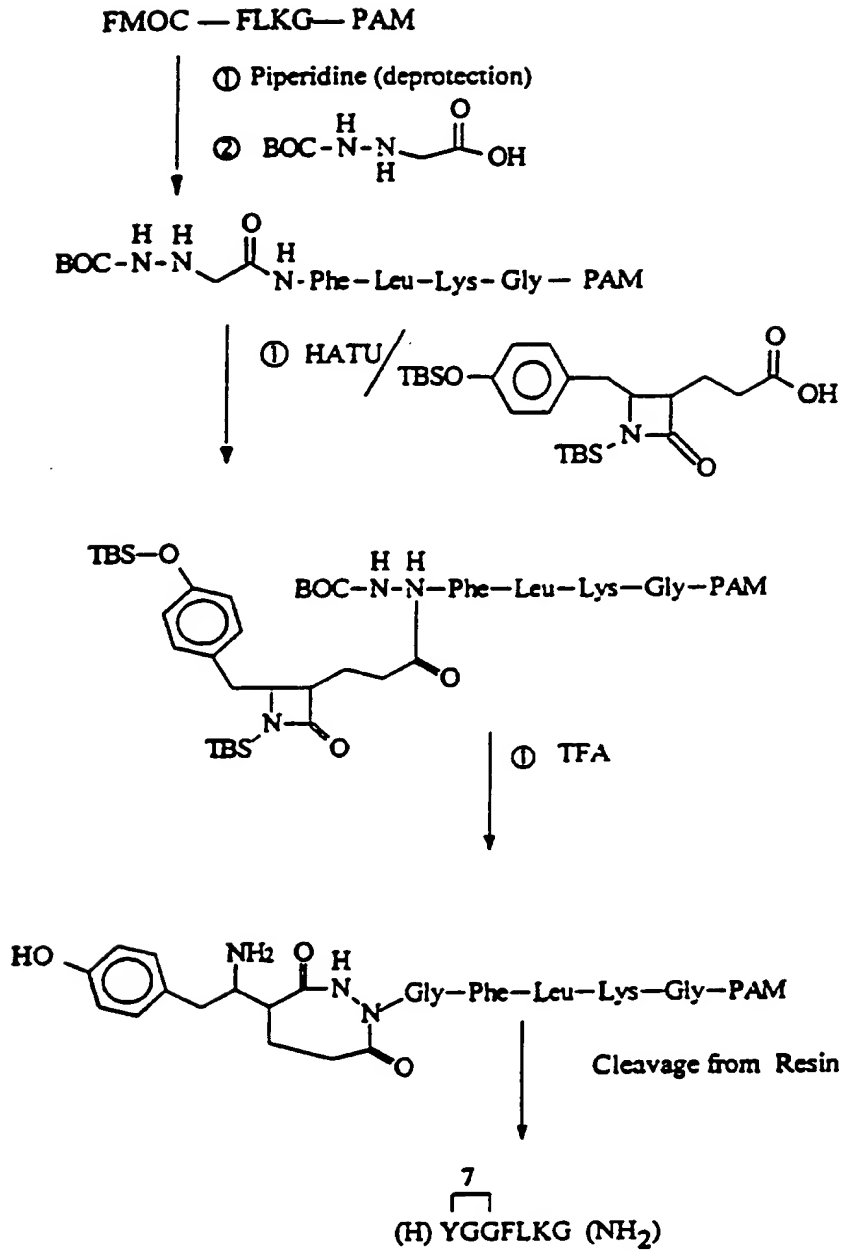

FMOC — FLKG— PAM
① Piperidine (deprotection)
② BOC-N-N
H H
OH
BOC-N-N
H H
N-Phe-Leu-Lys-Gly — PAM
① HATU
TBSO
OH
TBS
TBS—O
BOC—N-N—Phe—Leu—Lys—Gly—PAM
H H
TBS
① TFA
HO
NH2
Gly—Phe—Leu—Lys—Gly—PAM
Cleavage from Resin
7
(H) YGGFLKG (NH2)

FIG. 2B

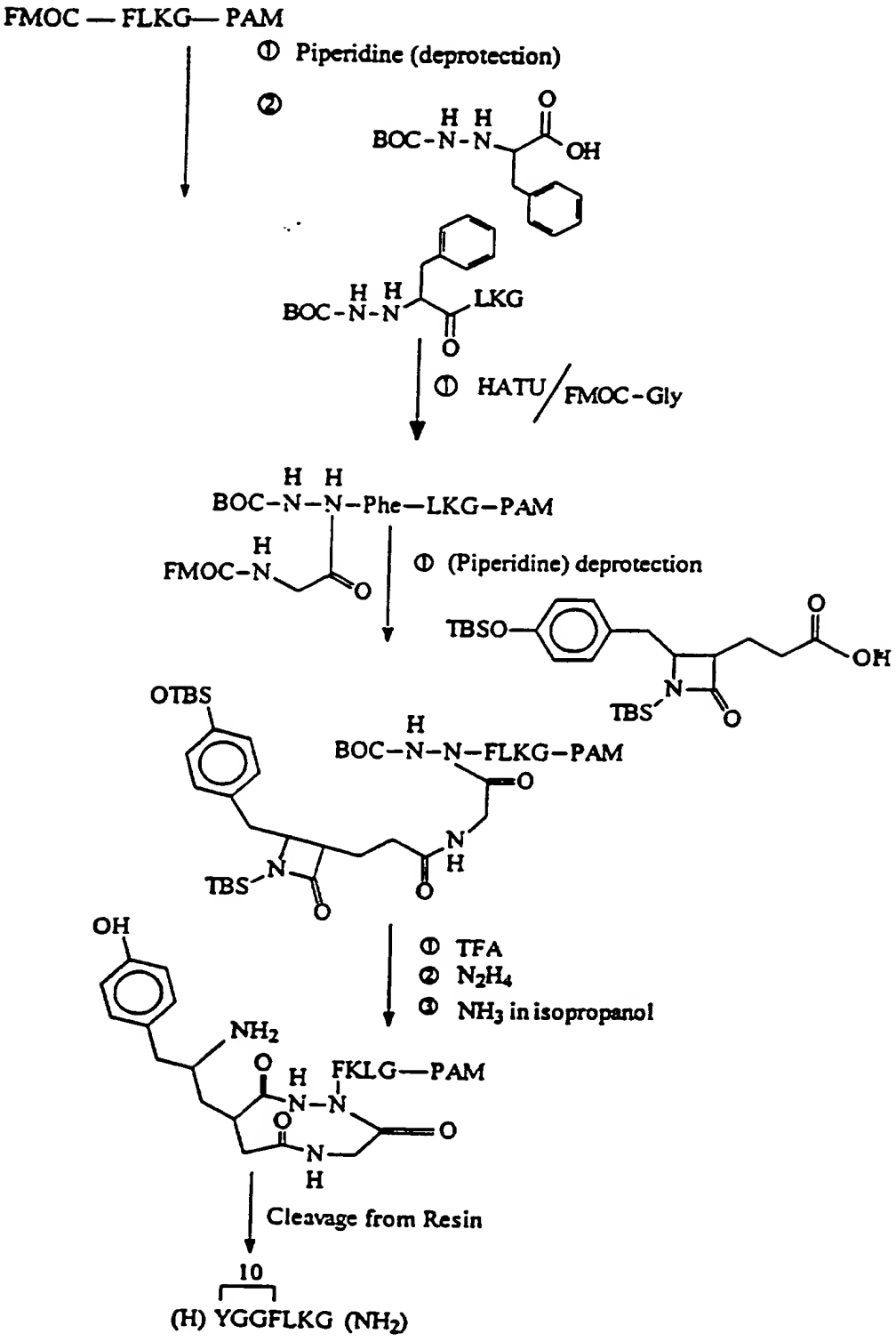

FMOC — FLKG— PAM
① Piperidine (deprotection)
②
BOC-N-N
OH
LKG
① HATU / FMOC-Gly
BOC-N-N-Phe—LKG-PAM
FMOC-N
① (Piperidine) deprotection
TBSO
OH
TBS
OTBS
BOC-N-N-FLKG-PAM
TBS
① TFA
② N_2H_4
③ NH_3 in isopropanol
OH
NH_2
FKLG—PAM
Cleavage from Resin
10
(H) YGGFLKG (NH_2)

FIG. 2C

INTERNATIONAL SEARCH REPORT

Int ional Application No
PC I/US 96/00786

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K1/04 C07K5/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,92 13878 (UNIVERSITY OF ILLINOIS) 20 August 1992 see the whole document ---	4-9
X	WO,A,94 03494 (UNIVERSITY OF ILLINOIS) 17 February 1994 see the whole document ---	4-9
X	JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, vol. 116, no. 25, 14 January 1994, DC US, pages 11580-11581, XP002004648 A A VIRGILIO & J A ELLMANN: "Simultaneous solid-phase synthesis of beta-turn mimetics incorporating side-chain functionality" see the whole document ---	1-3

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
3 June 1996	18.06.96
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Authorized officer Masturzo, P

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO,A,94 28028 (SELECTIDE) 8 December 1994 see the whole document ---	1-3
A	WO,A,94 08051 (COLUMBIA UNIVERSITY & COLD SPRING HARBOR LABS.) 14 April 1994 see the whole document ---	1-3
P,X	CHEMICAL ABSTRACTS, vol. 123, no. 11, 11 September 1995 Columbus, Ohio, US; abstract no. 144616m, D K CHALMERS & G R MARSHALL: "Pro-D-NMe-Amino acid and D-Pro-NMe-Amino acid; simple, effecient reverse-turn constraints" page 1353; XP002004649 see abstract & JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, vol. 117, no. 22, 1995, DC US, pages 5927-5937, -----	1-3

1

INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/US 96/00786

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9213878	20-08-92	AU-B-	1570292	07-09-92
		AU-B-	3066495	18-01-96
		CA-A-	2103577	08-08-92
		EP-A-	0573608	15-12-93
		JP-T-	6505486	23-06-94
		US-A-	5440013	08-08-95
		US-A-	5475085	12-12-95
WO-A-9403494	17-02-94	AU-B-	5000693	03-03-94
		CA-A-	2141447	17-02-94
		EP-A-	0656907	14-06-95
		JP-T-	7509723	26-10-95
		US-A-	5475085	12-12-95
WO-A-9428028	08-12-94	AU-B-	7048694	20-12-94
		CA-A-	2163637	08-12-94
		EP-A-	0705279	10-04-96
WO-A-9408051	14-04-94	AU-B-	5536994	26-04-94
		CA-A-	2143848	14-04-94
		EP-A-	0665897	09-08-95
		NO-A-	951230	30-03-95